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will be held
February 26 and 27, 1959
Kellogg Center, Michigan State University
East Lansing, Michigan

NATIONAL DAIRY COUNCIL
44th Annual Meeting, February 2-4, 1959
Statler-Hilton Hotel, Boston, Massachusetts

Annual Wisconsin
DAIRY MANUFACTURER'S CONFERENCE
January 28-29, 1959
Babcock Hall
University of Wisconsin, Madison

Call for papers for the 54th Annual Meeting of the American Dairy Science Association, to be held at the University of Illinois, June 15-17, 1959. See November Journal.

JOURNAL OF DAIRY SCIENCE

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PEOPLE AND EVENTS

Recent Deaths

Another of the "Elder Statesmen" in A.D.-S.A. history has passed from our midst. Professor Emeritus ANDREW BORLAND passed away on October 12, 1958 at his home in State College, Pa. following a long illness. He was 80 years and 4 months of age at the time of his death.

As the tenth president of our Association he served with distinction for two years, 1923 and 1924. Throughout his professional life (1910–1948) and following his retirement in 1948 he was closely identified with Association affairs serving on many committees including Journal Management. He served on this committee for twelve years and as its chairman for two years. Until his health failed in 1952 he seldom missed an annual meeting of the Association and still maintained a deep interest in the Journal and the Association until his death. The Association bestowed its highest honor, the Honorary Life Member Award to Professor Borland in 1948.

Former Journal Editor P. H. TRACY featured Prof. Borland in his column on "Pioneers in the Dairy Industry," *Journal of Dairy Science*, February, 1955, page 225. Because this account of Prof. Borland's life was so complete, parts of this article are appropriate here:

"As we judge the success of the leaders and pioneers of our industry, we often fail to appreciate the factors that contributed to their success, the sacrifices they have made or the depth of their contributions to society. Certainly this is true in the case of Professor Emeritus ANDREW A. BORLAND of The Pennsylvania State University.

A. A. Borland

"Professor Borland was born on a modest, but progressive, farm at Sandy Lake, Pa. He attended the public schools, but when the time came for college, he found himself without funds to further his education. In 1898 he became a public school teacher (country one-room school) at $30 a month, a position he held for 7 years. At 27 years of age and with a few dollars he had saved, he enrolled at The Pennsylvania State College, where he pursued the study of agriculture and soon became primarily interested in dairying. He found that room and board were expensive (board, $2.50 a week) so for the first year he prepared his meals in his room. He was fortunate in that the room was equipped with a steam radiator. From that radiator he attached a rubber hose to a double boiler arrangement (two tin cans) which made it possible for him to have "cooked" meals during the winter of 1905. He was given a highly-sought position as waiter in the college dining halls during his sophomore year, a position he kept until graduation.

"In 1909 he graduated with honors and was the recipient of the John W. White Scholarship ($400) for graduate study. With this scholarship, he went to the University of Wisconsin, where he completed his Master of Science degree in 1910. He then returned to Pennsylvania, accepted a position at Penn State as assistant in dairy husbandry, married Jessie E. Canon, and settled down to start his professional career in the field of dairy science.

"Young men of his ability and education were much in demand in those days, and in 1911 the University of Vermont convinced him that he should head its Animal and Dairy Husbandry Department. He served with notable success in that position for 4 years, at which time Penn State, a rapidly growing institution, asked him to return to be in charge of dairy husbandry extension. His four years in extension work, from 1915 to 1919, enabled him to become thoroughly familiar with the state's dairy industry and to become acquainted with its prominent personages. The following year he assumed the position of head of the Department of Dairy Husbandry and maintained it with distinction until his retirement in 1948.

"During his senior year in college, he entered an essay contest in agriculture. His essay entitled "Improving the Dairy Industry in Pennsylvania" was adjudged first, and he received the Barlow prize of $100 (five $20 gold pieces). The subject of this essay was the model upon which he devoted his life to the dairy industry.

"Professor Borland was always a very versatile and energetic worker, as well as an interesting speaker. When he became head of the Penn State Dairy Department, there was a staff of 4 men, a herd of 60 head of dairy cattle, a modest college creamery, and about 40 students studying dairying. During his tenure as head, the staff expanded to 15, a new modern dairy building was built, the herd increased to approximately 500 head, more than 1300 students were graduated, and a strong well-balanced research program was established. Because of heavy administrative and teaching responsibilities over the years, his personal research activities were limited

to a few outstanding pieces of work in the field of nutrition. Two of these, "The Effect of Low, Medium and High Protein upon Production and Health of Dairy Cows" and "Input and Output Relationships in Milk Production" are particularly noteworthy. He was always a strong advocate of research, surrounded himself with outstanding research scientists, and continuously sought support for their programs.

"As a teacher and counselor of men, he had few peers. His interest in students went far beyond the classroom, and many sought his advice long after graduation. His philosophy of education encompassed the "whole man" and placed much emphasis on character development and preparation for living as well as preparation for making a living. His students throughout the country hold him in high esteem and many credit their success to his friendly and sincere counsel."

Professor Borland's life was one of devotion to his profession, his family, his church and his community. He was one of the founders of the Westminster Foundation at State College and served as elder of the Presbyterian Church for 38 years. From his student days, when he was president of the Penn State Christian Association, he gave much attention and thought to the religious life of students regardless of creed. He was tolerant of all religious faiths and based his own life on sound Christian principles.

Professor Borland is survived by his wife JESSIE E. BORLAND, a daughter MRS. MARGARET E. BORLAND HARTZELL of Redlands, California, a son GERALD C. BORLAND of Westfield, Pennsylvania, and six grandchildren.

P. M. BRANDT, former head of the Dept. of Dairying at Oregon State College until his retirement on June 30, 1957, passed away on Oct. 3, 1958, at the age of 70 years. Prof. Brandt had been in poor health during the year prior to his death.

Prof. Brandt was born on Aug. 6, 1888, at Nevada, Missouri, and was reared in Missouri. He received his B.S. in Agriculture in 1910 and his A.M. degree in 1913 from the U. of Missouri. He held the position of secretary of the Missouri State Dairymen's Association from 1912-14. While working for his AM degree, he was an instructor in Dairy Husbandry and was promoted to assistant professor of dairy husbandry in 1913, then to assistant to dean and director and superintendent of Short Courses at the Univ. of Missouri.

In 1917, Prof. Brandt came to Oregon State College where he served on the staff for 40 years. His record is outstanding both in the advancement of the dairy industry as well as in civic affairs. Mr. Brandt served as president of the original Oregon Dairy Council which was organized about 1920. He was a member of the City Council from 1924-33, elected Mayor of Corvallis 1933-34 and served diligently on various State committees. He served as President of the Corvallis Chapter of the Oregon State Employees Association and as the director of retirement and also vice-president of the state association. He was assigned to numerous committees for the American Dairy Science Association and the Western Division of the American Dairy Science Association during his tenure at O.S.C. Among Mr. Brandt's better known publications were policy statements prepared for special occasions, "The Dairy Industry at the Crossroads," January 17, 1945, and "Guide Posts for the Dairy Industry," January 8, 1954.

Prof. Brandt fostered research related to both dairy production and dairy processing. Studies included brucellosis control, butter marketing, milk production costs, artificial breeding of cattle, bovine reproduction with low-carotene rations, relation of milk quality to equipment, buildings, and production methods, irrigated pastures, ensiling methods, improvement in manufacturing, processing and packaging of butter, cheese and ice cream, and research dealing with the normal composition and re-evaluation of cows milk.

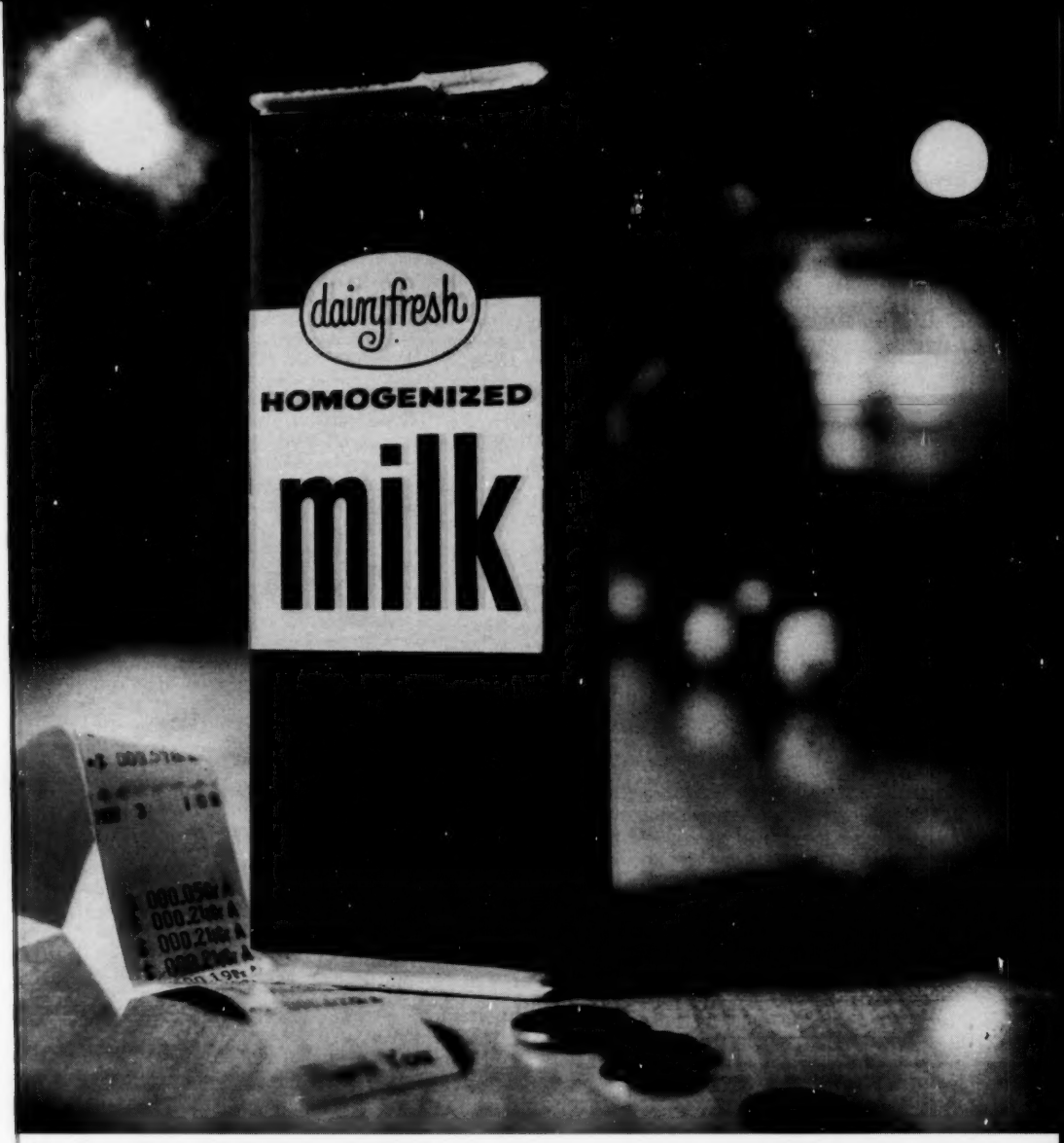
Prof. Brandt was a member of Gamma Sigma Delta, Sigma Xi, American Dairy Science Association, and American Society of Animal Production, Masonic fraternities and Sons of the American Revolution.

Mr. Brandt is survived by his wife, MATTIE MAY TORR BRANDT, a son PHILLIP M. BRANDT, JR., Salem, Oregon, a daughter, MRS. HARRIETT ANNE HUTCHENSON, North Ridge, California, and five grandchildren.

To The ADSA Membership

The 1958 annual meeting of the Dairy Remembrance Fund authorized a contribution sufficient to cover the cost of 12 copies of the recruitment film, "This is the Dairy Industry," for distribution through Modern Talking Pictures Service and for showing to thousands of secondary schools throughout the nation as part of the project initiated by the Dairy Industry Committee whose constituents guaranteed the distribution of a total of one hundred copies of this film. Our contribution has been made and this phase of the educational program is under way. We believe that it will be of invaluable assistance to our dairy colleges.

The meeting, also, authorized making contact with each one of the agricultural colleges throughout the United States and territories. The purpose of this was to inform the colleges of the availability of the recruitment film and, also, to tell them that the Fund would cooperate by furnishing up to 50% of the cost or through matching industry funds for the balance needed where the college could make a



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partial payment from its regular budget. We have a considerable volume of correspondence with many of the colleges and are delighted to report that the Fund has assisted in making deliveries to the following institutions: Univ. of Florida; Rutgers Univ.; Texas Technological College; Connecticut State Univ.; Oregon State Univ.; Colorado State Univ.; Univ. of Georgia; Texas A. & M.; North Carolina State College; Univ. of California; Cornell Univ.; Louisiana State College; Fresno State College.

Negotiations are still under way on behalf of several other State colleges. In addition, we were instrumental in providing the information which enabled several other colleges to purchase the film with their own funds. We also found that a half dozen colleges had either obtained a copy of the film already through local dairy groups or were having copies made available on a loan basis from such groups. We believe that we have greatly assisted in bringing industry groups closer to all of these colleges with whom we have been working and that this project has brought a great deal of good will to the Fund. In all cases where industry groups worked with us by making contributions toward the cost of the film, we have invited the trade groups to become sponsors through their better knowledge and the type of work which we can do together.

The annual meeting also authorized the making of three assistance loans in the amount of up to $400 each for worthy students who were faced with an emergency situation. One such loan has recently been granted to a student at Iowa State College through the recommendations of the head of his department. The student had an outstanding record and was adjudged to be of great future value to the dairy industry. His father had passed away and his mother, who had taken employment, had lost her position and the young man was in serious financial straits. A note in the amount of $350 is in our possession. No payment will be asked for until two years after his expected graduation in 1960. We are charging only 2% interest and will credit 1% of this provided the young man remains in the upper portion of his scholarship group. This is the first such loan granted by the Fund and it will be interesting to await developments. We have had indications that one or two other requests may be forthcoming during the present college year.

The survey reported to the annual meeting brought out the fact that there was no central source of information to which either the industry or the colleges could turn for accurate knowledge of the numbers of students registered in various categories. The meeting authorized an exploration, preferably with the American Dairy Science Association, which would lead to the establishment of a central facility where such information could be reported by the dairy educational institutions at least once a year. It was expected that this would greatly assist the colleges themselves and also the industry. It could help to establish a better knowledge of the financial needs and of the recruitment needs and, also, the employment needs for both undergraduates, graduates and researchers. There has been an exchange of letters with officials of American Dairy Association. Its Committee on Education has been given authority to study the matter and to make a proposal to the Dairy Remembrance Fund in the near future. We are awaiting receipt of this proposal. When it is in hand and has been analyzed, there will have to be a discussion of it by the Board of the Dairy Remembrance Fund which will probably wish to call in representatives of national dairy trade associations in order to implement the establishment of the proposed facility.

Oregon's Wilster Retires

G. H. WILSTER, professor of dairy manufacturing at Oregon State College for 29 years, officially retired last July 1.

Dr. Wilster was born in Denmark in 1893 and received his early education in that country. In 1911 at the age of 18 he went to New Zealand and began his career in the dairy industry. He came to the United States in 1914 and enrolled immediately at Iowa State College where he received his B.S. and M.S. degrees. After several years at Utah State Univ. and industrial positions, he again returned to Iowa State College and secured his Ph.D. degree in 1928. He accepted a position at Oregon State College in 1929 and remained there until his recent retirement.

G. H. Wilster

Dr. Wilster was instrumental in improving the quality of Oregon Dairy products through a monthly scoring program for butter and through the educational programs of Oregon Dairy Industries Association. He served continuously as secretary of this organization from 1929 until 1958. He was honored for his long service to this organization at the Oregon Dairy Industries 47th Annual convention in Corvallis last February when a life membership was awarded him.

Dr. Wilster was internationally known for his research work on butter, cheese and concentrated milk products. In 1945 he spent a year's sabbatical leave working on dried milks at the University of Illinois. He attended the 12th International Dairy Congress in Stockholm in 1949 as an official United States delegate. One of the high-lights of his career was his selection as a Fulbright lecturer at the

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Danish Royal Agricultural College, Copenhagen in 1955–56. During his stay in Denmark he also lectured in several neighboring European countries under the sponsorship of the U. S. Department of State.

Some 206 publications bear Dr. Wilster's name as author or joint author including three widely used text books.

Dr. and Mrs. Wilster now reside in Lakeside, San Diego County, California. Upon leaving Oregon, Dr. Wilster hinted that he may soon accept a foreign assignment.

Cornell News

Over 250 scientists and industrial representatives from the United States and Canada attended a conference on Fundamentals and Scientific Advances in Cottage and Cheddar Cheese Making at Cornell Univ., Oct. 7–9, 1958.

They listened to talks on the shattering of cottage cheese, practices for keeping cheese fresh, manufacture of cottage cheese from spray-dried milks and proper packaging and sanitizing techniques. New scientific techniques in labelling milk and cheese with radioactive isotopes to better understand cheese ripening were discussed.

The highlight of the three-day meeting was a full afternoon's discussion devoted to cheese mechanization. The first formal presentation of a fully mechanized cheese making procedure, Ched-O-Matic, was received with much interest. On the last day of the meeting the entire group went to northern New York to see a very successful demonstration of the Ched-O-Matic method. A visit to one of the most modern cottage cheese plants in the nation was included in this tour.

Another interesting development at this Cornell meeting was the formation of a new dairy industry organization, the American Cottage Cheese Institute. At a dinner held by this group at Statler Hall, the objectives and the names of sixteen directors were announced for the next year.

The representatives attending the Cheese Conference were guests of the Cornell Dairy Science Club at a Smoker at the Cornell Residential Club, Tuesday evening, October 7. As a special treat the club served an assortment of domestic and foreign cheeses.

The Dept. of Dairy Industry was host on Oct. 6, 1958 to the New York State Sanitarians' Committee on Educational and Professional Improvement. In addition to the committee members other leading members of industry, regulatory agencies and educational institutions were special guests.

W. M. FARNSWORTH of the N. Y. State Agricultural and Technical Institute at Delphi, N. Y., chairman of the committee, presided. He pointed out that the meeting had been called to discuss the declining enrollment in the dairy industry curriculum at most educational institutions.

F. M. SKELTON, General Ice Cream Division, National Dairy Products Corporation discussed the problem from the industry viewpoint. R. F. HOLLAND, head of the Dept. of Dairy Industry, Cornell Univ. and W. F. KENNAUGH, head of the Department of Agriculture, N. Y. S. Agricultural and Technical Institute presented the academic viewpoint.

Mr. Farnsworth then opened the meeting for questions and a discussion of the views presented. As a result of the discussion it was proposed and unanimously agreed that a committee of industry representatives be appointed to inform industry of the problem and to arrange for the dissemination of information on career opportunities and positions in the dairy industry to guidance counsellors and high school students. Chairman Farnsworth appointed JOSEPH CACIOPPO, Grandview Dairies, Arkport, N. Y. to head this new committee. Mr. Cacioppo was asked to select his committee members.

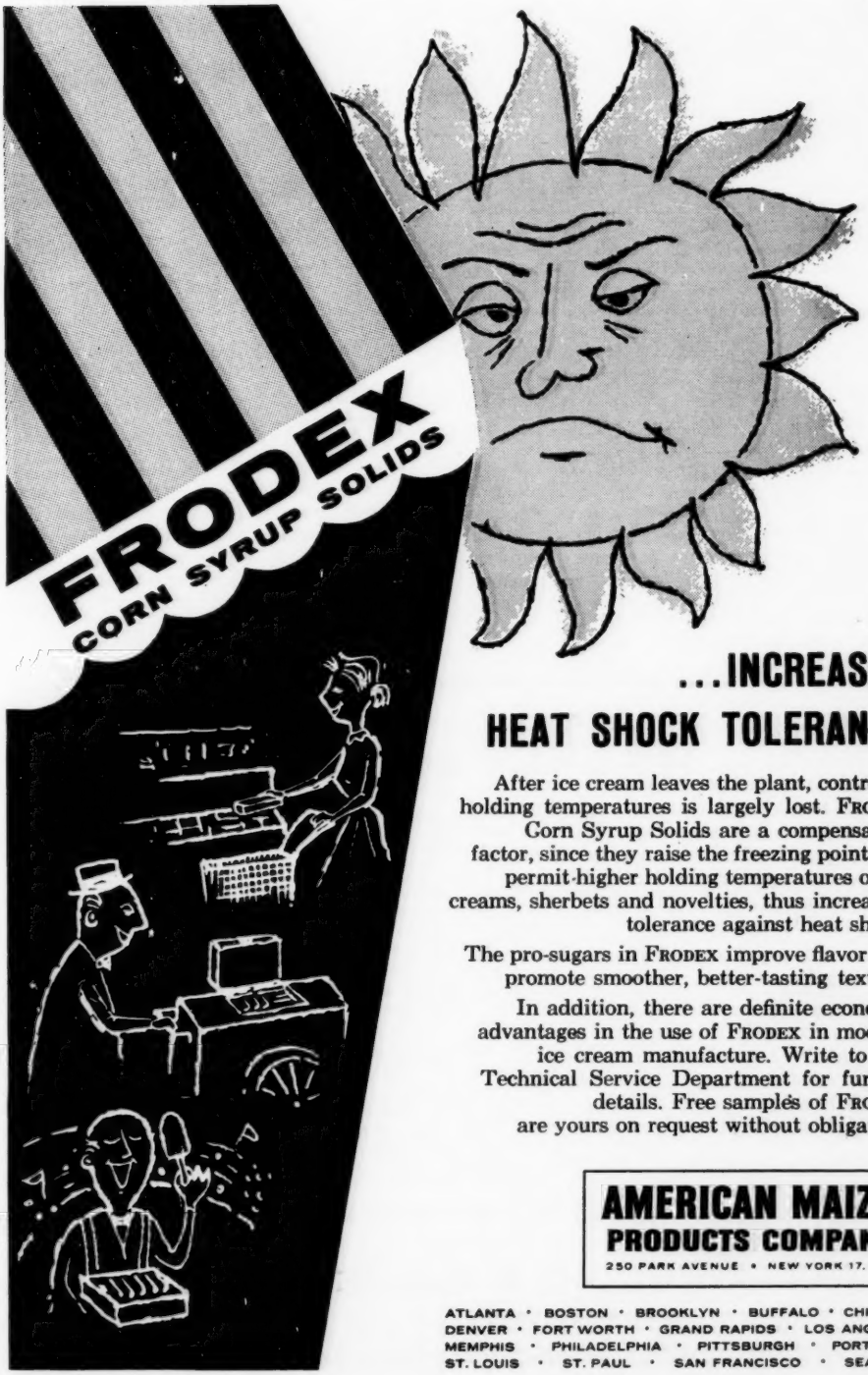
A booklet entitled, "Opportunities for Graduate Study in Bacteriology and Dairy Science" has been prepared recently by the Department of Dairy Industry at Cornell Univ. Copies of this booklet may be obtained by writing to the department.

A brand new national organization to promote cottage cheese—The American Cottage Cheese Institute — has been established here. Groundwork was done for the new organization Oct. 8 when 150 cottage cheese manufacturers met at Cornell Univ. to discuss fundamentals and scientific advances in cottage cheese making.

For many years, industry spokesmen said, there has been a need for an organization to: (1) Be a clearing house for manufacturing and marketing information; (2) Help solve production problems in general; (3) Seek greater consumer acceptance of cottage cheese.

At the first annual dinner held on the Cornell campus, Chairman ERIK LUNDSTEDT of Hood and Co., Boston, appointed D. K. BANDLER, Ithaca, N. Y. Executive Secretary of the Institute and announced that temporary offices would be located at 50 Warren Hall on the Cornell Campus. Lundstedt presented an 11-point program to guide the Institute and said that copies of the by-laws are available at the Ithaca office.

Sixteen directors were elected to serve one year terms during the organizational phase of the Institute. The officers are Lundstedt who is Director of New Product Research for Hood, chairman; HENRY LEBER, technical supervisor, Manufacturing Division, Dairymens' League



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Cooperative, Syracuse, vice-chairman; ZOL ZAUSNER, President of Meyer Zausner and Co., New Holland, Pa., secretary; and E. F. CROWLEY, Crowley Milk Co., Inc., Binghamton, N. Y., Treasurer.

Other Directors include NEIL ANGEVINE, St. Louis, Mo; WILLIAM BAULKWILL, New York; JOSEPH BILTEKOFF, Buffalo; JOSEPH CACIOPPO, Brooklyn; LARRY CREIGHTON, Elmira; E. S. GUTHRIE, Ithaca; WALTER HILDEBRANT, Enosburg Falls, Vt.; C. B. LANE, Walton; HENRY LEHMKUHL, Rochester; GEORGE MARTIN, Honeoye Falls; FREDERICK SKELTON, Schenectady and THOMAS C. SHOTWELL, Chambersburg, Pa.

The new Institute outlined its objectives as follows:

(1) An Institute dedicated to the advancement of the Cottage Cheese Industry.

(2) An Institute staffed by experts and consultants in the manufacture of Cottage Cheese.

(3) A Summer Seminar conducted by Institute staff and University scientists for the purpose of teaching members the latest in the art of Cottage Cheese making and exchanging ideas for the mutual benefit of everyone concerned.

(4) A center of information and prompt aid in scientific, technical, nutritional and merchandising matters for its membership, affiliated groups and press.

(5) An Industry supported Research and Production Laboratory serving its membership in all phases of the manufacture of Cottage Cheese.

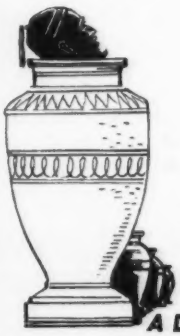
(6) An Engineering Laboratory supported by the Dairy Industry suppliers, set up for the testing of equipment, packaging machinery, automation devices, packaging material, design and correct labeling for the purpose of obtaining The American Cottage Cheese Institute's stamp of approval for its members.

(7) A statistical Bureau where accurate information can be obtained on the production and sale of soft cheeses in the United States, and other information of statistical importance, like consumer preference, prices, etc.

(8) A Standards Committee, representing the membership, to work on quality and sanitation requirements necessary to obtain the Institute's seal of approval on packages for members who qualify.

(9) An Institute representing the industry in matters pertaining to Federal, State and local governments. An Institute which effectively, through its membership representation, can promulgate with Federal agencies regulations for controlling the quality of milk used in Cottage Cheese (antibiotics, etc.).

Ali Baba *and the* 40 *Thieves*



The fabled Ali Baba never suspected that he was harboring 40 armed thieves under his roof. The fable states that Ali's watchful servant, Morgiana, discovered them lurking there and saved Ali and his valuables.

There are 40 thieves lurking in every business operation today — awaiting every opportunity to rob it of profit. There are Wastes, Production Losses, Distribution Costs and a host of others — most of them well entrenched and not easily recognized.

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The Bureau uses a sharp and practised eye for ferreting out of Milk and Ice Cream operations the 40-odd leaks that may be robbing them of hard-won profit.

If you suspect **you** are paying tribute to some of these hidden thieves, why not join the Bureau **now**? Avail yourself of the Bureau's many services which will put your operation back on the road to **full** profit. For complete information, write

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(10) An Institute which, in integrity, honesty and sincerity of purpose, will demand the confidence and respect of our health authorities, industry and consumers of Cottage Cheese.

(11) A magazine — The American Cottage Cheese Journal — A semiannual journal containing information of value to the industry.

California Tech News

TOM NUNES of San Rafael, a sophomore student in dairy husbandry at California State Polytechnic College, was top individual judge at the intercollegiate judging contest just completed in connection with the Grand National Livestock Exposition in San Francisco's Cow Palace. Nunes was high scoring judge among 27 contestants from nine colleges and universities.

In the team competition Cal Poly placed second although rating first in the judging of Ayrshires and second in judging both Holsteins and Guernseys.

Nunes won with one of the highest scores ever made in judging competition at the Cow Palace. He was first in Jerseys, Brown Swiss and Guernseys and third in Ayrshires.

W. R. GOMES of Hanford was top judge of Ayrshires and second in Jerseys. PAUL SULLIVAN of Eureka, third member of the Cal Poly dairy judging team, was seventh in Holsteins.

Retiring the Grand National Livestock Exposition's Bank of America judging trophy with a third straight win California State Polytechnic College's livestock judging team set its sights for the Golden Spike Exposition at Ogden, Utah, Nov. 15.

The Cal Poly home campus team won the swine division, placed second in cattle, sheep and horses at San Francisco in a field which saw the San Francisco exposition draw a record number of participating teams. Eleven universities and colleges from inter-mountain, northwest and Pacific coast states were represented.

Member of Cal Poly's trophy-retiring team was LILLA HUNTER, animal husbandry major from Hollister, and first coed ever to compete on a Cal Poly judging team. She turned in a top record with first in horses and second in beef cattle and was second in over-all rating for the entire contest.

In addition to the championship trophy, now permanently in Cal Poly's collection, the team was awarded the American Hampshire Swine Registry's trophy as top performer in the swine division.

In addition to Miss Hunter, Cal Poly team members included: AARON NELSON, El Cajon, who ranked fifth overall and second in horses; JAMES CODY, La Mesa, third in horses and eighth in overall; DAVID RICKANSRUD, San Luis Obispo, second in sheep and seventh overall; KEN WHIPPLE, Alama, Nevada. DON TOMPKINS, King City, served as first alternate. Team coach is animal husbandry faculty member RICHARD JOHNSON.

A new feature of this year's exposition contest was presentation of the team at the evening show where a capacity Cow Palace crowd saw each team member presented individually by the master of ceremonies.

ROLLO A. BEATY, San Francisco, president of the California Dairy Council and chairman of the research committee of the Dairy Advisory Board, is California State Polytechnic College's "Alumnus-of-the-Year" for 1958.

Announcement of the nationally-known dairy figure's selection was made by HERBERT PEARCE, Long Beach, a Douglas aircraft engineer who is national president of Cal Poly's alumni association. Beaty received "Alumnus-of-the-Year" honors at Cal Poly's home campus Homecoming celebration November 8.

Graduating from Cal Poly in 1917, Beaty began his long activity with the dairy industry when he joined the Golden State organization in 1923. From 1932 to 1945, he was with that organization in Los Angeles and participated in the early efforts to stabilize the dairy market.

In 1945, he was moved to San Francisco and became a director and later president of the Creamery Operators Association. He also served as a member of the Producer Relations Committee of the Milk Industry Foundation and in 1952 was made a vice-president of Golden State. During that period he became a director of the Dairy Institute of California and was appointed to the Dairy Advisory Board in 1952. He was chairman of the institute's legislative committee during the 1953 legislative session.

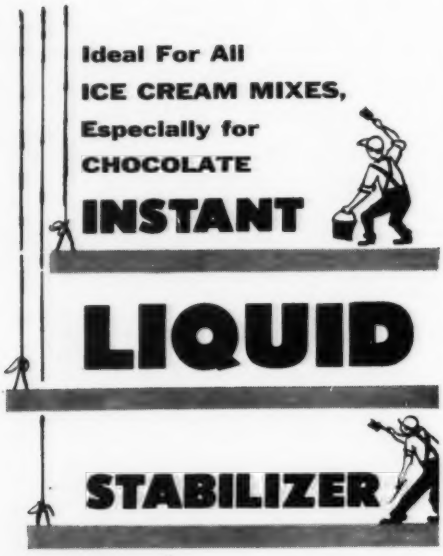
When Golden State became a part of Foremost Dairies this year, Beaty was assigned to that company's general staff and handles product procurement for Pacific Coast operations. In addition to other honors, Beaty will be specially recognized at Cal Poly's Homecoming football game with Montana State.

Ohio State News

"What Industry Wants — Industry Will Get," is the philosophy underlying the adult education program of the Dept. of Dairy Technology, Ohio State Univ., Columbus.

To determine the desires and needs of industry, the department this past summer made a comprehensive survey of the dairy companies of Ohio. The study was designed to determine whether or not the dairy industry of Ohio was interested in short courses and conferences; and if so, what specific types of programs should be developed.

Responses indicated that the industry was not interested in two-week short courses in



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market milk and ice cream, was not particularly interested in correspondence courses in ice cream, was mildly interested in a short course in market milk, was moderately interested in a conference on personnel, and was highly interested in a conference dealing with research interpretations for management.

On the basis of this study, the department is re-designing its entire adult education program.

Also, as a result of the study, the department has discontinued its offerings of two week short courses and correspondence short courses for this year. In addition, it has taken steps to re-design the format of the Annual Dairy Technology Conference in order to come up with a program that will meet more fully the current needs in industry.

Another result of the study is that the department is starting on a newsletter approach to keep dairy plants of the state informed about new developments in pure and applied research.

W. J. HARPER of the Dept. of Technology presented a paper on "The Factors Affecting the Loss of Antibiotic Activity in Milk" before the annual Antibiotic Symposium, Washington, D.C., Oct. 15-17.

The November meetings of the four Ohio Dairy Technology Societies — Maumee Valley, Northeastern Ohio, Central Ohio, and Cincinnati—were designated "High School Student Night."

In order to create career interest in the dairy industry, members of the societies invited young high school men to their meetings. The students were welcomed by the chairman of the education committees who also spoke briefly on career opportunities in the dairy industry. High school information packets were distributed among the students.

Main speaker was FLORIAN SOLZAN, Edward B. McLain Co., Memphis, Tenn. His topic was "Plant Cost Control."

Twenty-two scholarships have been made available to students majoring in dairy technology for the year 1958-59. The scholarships are freshmen (14), sophomores (2), juniors (3), and seniors (3).

Sponsors for the scholarships include the Babcock Dairy Co., Toledo; Beatrice Foods, New Bremen; Broughton's Dairy Farm, Marietta; Central Ohio Dairy Technology Society, Columbus; Columbus Milk Distributors Association, Columbus; Dairy Milk Foundation, Dayton; Esmond Dairy Company, Sandusky; Hopewell Dairy Company, Bellefontaine; Maumee Valley Dairy Technology Society, Toledo; Meyer Milk Products Co., Cleveland; North-

eastern Ohio Dairy Technology Society, Cleveland; Sealtest Ohio Division of National Dairy Products Corp., Cleveland; Stolts Memorial Fund Scholarship, Columbus, and the Warren Sanitary Milk Co., Warren.

L. R. STAUFFER, president of the Warren Sanitary Milk Co., Warren, has been named the 1958 recipient of the Dept. of Dairy Technology Award of Merit.

The Award, which is given for outstanding leadership in the Ohio dairy industry, was presented at the department's annual Homecoming Brunch, Oct. 25, by L. L. RUMMEL, Dean of Agriculture at O.S.U.

"Making Dollars with Sense" is the theme of the 26th Annual Dairy Technology Conference which will be held at O.S.U., Feb. 3-6, 1959.

This year the format of the conference is being changed markedly and special sessions will be developed in the following areas: (1) field problems; (2) technical control; (3) cleaning and sanitation; (4) dairy engineering; (5) cultured milk products; and (6) management and plant operations.

Special emphasis will be placed this year on management problems and ways and means of reducing costs in all phases of dairy plant operations. An outstanding group of speakers is being obtained for the program. Wherever possible, the program will be designed along a problem-solving basis, using a clinical approach.

Once again, the four Ohio Dairy Technology Societies are cooperating with the department in arranging for the annual banquet which is held in connection with the conference. One of the nation's outstanding speakers, HARRY ("Hap") BOWSER, sales development manager of Dairypack, Inc., will deliver the banquet address.

News from North Carolina

W. E. THOMAS has been appointed head of the dairy husbandry section of the Animal Industry Dept. succeeding J. E. LEGATES, who has been acting head since R. K. WAUGH resigned.

Dr. Thomas, a graduate of the Univ. of Kentucky, completed his doctorate at Cornell Univ. in 1950, after which he joined the staff at Kentucky in the Animal Industry Dept. He came to North Carolina State College in 1953, where he has devoted much of his activities to research and teaching in Ruminant Physiology.

Dr. Legates has been named head of the animal breeding section, which is a newly established section in the Animal Industry Dept. He will direct teaching in animal breeding as well as research in dairy cattle, beef cattle, swine and sheep breeding. Also, incorporated in the new section will be reproductive physiology research and teaching.

E. J. STONE, Assistant Professor in Dairy husbandry at Louisiana State Univ. is spending one year on sabbatical leave at North Carolina State College where he is doing graduate work toward a Doctorate in Dairy Husbandry. He is working in the field of forage utilization by ruminants.

S. T. MUSGRAVE, professor in the Dairy Dept. at Oklahoma State Univ. is spending six months on sabbatical leave working with J. E. LEGATES in the animal breeding section. He plans to return to Oklahoma in January 1959.

Clemson News

J. J. JANZEN has been named associate professor of dairying and associate dairy scientist on the staff of the Clemson Agricultural College and the South Carolina Agricultural Experiment Station.

Dr. Janzen was formerly with the Continental Can Co., Central Research and Engineering Division, Chicago. He received his B.S.A. degree from the Univ. of Manitoba, Canada, and his M.S. and Ph.D. degrees from the Univ. of Wisconsin.

His new duties will include teaching, research, and coaching dairy products judging teams.

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Ontario News

D. H. BULLOCK recently rejoined the staff of the Dept. of Dairy Science at the Ontario Agricultural College, Guelph.

After three years' service in the R.C.A.F., Dr. Bullock attended O.A.C. and graduated in the Dairy Science Option in 1950. He then spent two years working for New Lawson Dairy Products Ltd. as plant superintendent. After returning to O.A.C. as a member of the staff of the Dept. of Dairy Science, Dr. Bullock received his M.S.A. degree in 1955. In 1956 he was granted a leave-of-absence to attend the Univ. of Wisconsin for further graduate studies. He recently was granted his Ph.D. degree from Wisconsin.

His research concerned the reconstitutability of whole milk powder and was directed mainly towards a new form of dried whole milk which is dried in films under high vacuum and at relatively low temperatures. His findings are the basis of a patent application by the Wisconsin Alumni Research Foundation. Dr. Bullock's duties at O.A.C. include teaching concentrated and dry milk products and director of research for the Dairy Science department.

D. C. CHUNN joined the staff of the Dept. of Dairy Science, Ontario Agricultural College in March 1958 as a lecturer. He is a graduate of the Univ. of Manitoba in 1949 and since his graduation has been employed in the Dairy Industry in Ontario.

Before joining the O.A.C. staff he held positions as chemist, plant superintendent and assistant manager with Quinte Milk Products, a plant which manufactures a complete line of dairy by-products.

Mr. Chunn also spent a year with the Ontario Dept. of Agriculture doing research on milk flavours, cream sampling and cream sample containers. Mr. Chunn is lecturing in Butter Manufacture and Plant Management.

Oregon News

E. A. DAY has recently joined the Dept. of Food and Dairy Technology staff at Oregon State College as an assistant dairy technologist. Day obtained his B.S. degree from Univ. of Maryland and did his graduate work at Pennsylvania State Univ., receiving his Ph.D. degree in 1957. He served on the staff for two years at the Univ. of Maryland before coming to Oregon. He is currently engaged in research dealing with the chemistry of milk flavors.

F. G. HUETER has joined the staff of the Dept. of Dairy and Animal Husbandry at Ore-

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4. Conduct research on new ideas suggested by you.

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gon State College as an assistant dairy husbandman. Hueter is a native of Maryland and received his B.S., M.S. and Ph.D. degrees from the Univ. of Maryland, obtaining his terminal degree in June of this year. He is currently engaged in research work in rumen physiology and nutrition.

Maine News

Dairy operators and managers from the state of Maine met in October at Augusta to organize an association to work for the welfare of the dairy industry. The new group was named the "Maine Dairy Technology Associates."

The first slate of officers for the new group includes DWIGHT STAPLES, Old Tavern Farms, Portland, president; EDWARD DENNY, Round Top Farms, Damariscotta, vice president; KENNETH JOHNSON, Chief of Dairy Inspection, Maine Dept. of Agriculture, secretary; and GORDON RAMSDELL, associate professor of dairy science, Univ. of Maine, Orono.

Wisconsin Dairy Industry Conferences Scheduled

Two dairy industry conferences have been scheduled at the University of Wisconsin in January—in conjunction with the 1959 Farm and Home Week.

The Wisconsin Dairy Manufacturers' Conference will be held Jan. 28-29. The Dairy Fieldmen's Conference will be held the afternoon of Jan. 29 and the morning of Jan. 30.

The manufacturers will discuss selling nonfat dry milk, dairy technology and product accountability, enzymes and heat treatments of market milk, functions of electronics, additives in the ice cream industry, and mechanization of cheesemaking.

The fieldmen will consider farm inspections, quality control with bulk tank milk, and new methods of measuring milk quality for intrastate shipments.

Speakers from different parts of the country will appear on the programs. The Wisconsin Dairy Technology Society will hold a dinner meeting Jan. 29.

P. L. Day Appointed by FDA

Appointment of P. L. DAY, biochemist of the Univ. of Arkansas School of Medicine, to the new position of Scientific Director of the U. S. Food and Drug Administration was announced today by G. P. LARRICK, commissioner of food and drugs.

"This position has been established to strengthen the scientific organization of the FDA," Mr. Larrick said. "The Food and Drug Administration faces new and highly complex scientific problems. Dr. Day has a distinguished record in the organization and conduct of research programs. He will be responsible for maintaining the scientific quality of our work and for coordinating all of our research efforts, both in Washington and in our field laboratories across the country."

Mr. Larrick said that the existing organization of FDA's Bureau of Biological and Physical Sciences in Washington will not be disturbed. R. S. ROE, Director of the Bureau, will continue to direct the programs of the Bureau. Dr. Day's assignment, Commissioner Larrick explained, will be to work as the staff arm of the Commissioner in the formulation of broad scientific policy. He will also work directly with FDA scientists in the designing of experimental programs to achieve the objectives of the Food and Drug Administration.

Dr. Day has been with the Univ. of Arkansas School of Medicine for the past 31 years, during which it has become an important center of medical research. He leaves the positions of professor and head of the Dept. of Biochemistry and assistant dean of the graduate school.

Dr. Day is the author or co-author of more than 100 research papers in the fields of biochemistry and nutrition. He is a member of numerous scientific societies, boards, and committees, including the American Society of Biological Chemists; American Institute of Nutrition; American Chemical Society; Society for Experimental Biology and Medicine; Alpha Omega Alpha (honorary medical society); the Southern Society for Clinical Research, and the American Association for the Advancement of Science. He is also a member of the Food and Nutrition Board and the Institute of Laboratory Animal Resources of the National Research Council, and has been a member of the Biochemistry Committee, National Board of Medical Examiners, and Scientific Advisory Committee of the National Vitamin Foundation.

He obtained his A.B. degree from Willamette Univ., Salem, Oregon, in 1921, his M.S. degree from Columbia Univ. in 1923, and his Ph.D. degree from Columbia Univ. in 1925. Dr. Day will assume his new position with the Food and Drug Administration sometime before the first of the year.

Progress Reported in Basic Ag Research

Looking forward to the day when basic research will comprise 50 percent of the projects in Agricultural Research Service, B. T. SHAW, administrator, reported recently the first year's progress toward new discoveries by the pioneering research laboratories of the U.S.D.A. Among the advances he cited were:

(1) New information on how nutrients get from the soil into plant root cells—knowledge that is basic to an understanding of plant growth (and perhaps of animal growth as well, since the cellular mechanism involved may be similar in both animals and plants).

(2) Insight into the process by which antigens—substances in animal blood that may

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hold the key to better livestock breeding—are formed in blood cells.

(3) Progress in finding out how the clock-like mechanism in plants, responsive to light, tells seeds when to germinate, flowers when to bloom, and trees when to stop making leaves and develop buds.

"The pioneering laboratories," stated Dr. Shaw, "recognize the fact that it is important to the working scientist that some funds be allocated for support of research that is aimed, not at specific, practical problems or objectives, but rather at the advancement of science. Only in this way can he have the freedom he needs to follow where the research trail leads and find the unlooked for. These laboratories are a response to the need for the freer play of genius in agricultural research."

Educational Conference on Training Students for the Food Industry

A conference for educators and leaders in the food industry was held in May at the Univ. of Illinois for the purpose of discussing and formulating a training program for students in food technilogy. Forty-two invited people attended the 3-day session.

A model curriculum was unanimously approved by the industry representatives. The subjects were classified as follows:

Subject	Semester hr.
Chemistry:	
Inorganic	5
Qualitative	5
Quantitative	5
Organic	5
Physical	8
Food Analyses	4
Biochemistry and Nutrition	6
Mathematics:	
Algebra	3
Analytical geometry	3
Calculus	8
Biology	5
Microbiology	5
Food bacteriology and sanitation	5
Physics	10
Food Processing	12
Unit operations	10
English	6
Speech and technical writing	3
Economics	3
Humanities and Social Sciences	12
Physical education—Military	8
	131

USPHS Courses in Cincinnati

Two courses will be offered Feb. 2–11 by the Public Health Service's Robert A. Taft Sanitary Engineering Center in Cincinnati.

The first course is "Microbiological and Chemical Examination of Milk and Dairy Products" for bacteriologists in state, munici-

pal, and other laboratories engaged in milk analysis and dairy products examination.

Theory, laboratory techniques, and interpretation of tests used in evaluating the sanitary quality of milk and dairy products are considered, with emphasis on selection and application of procedures outlined in *Standard Methods for Examination of Dairy Products* and on certain screening tests. Laboratory practice includes procedures used in detection of bacterial groups or of species of special significance in the quality control of milk.

The second course is "Laboratory Methods for Prevention and Control of Food-Borne Disease," to be offered Feb. 9–11 for laboratory supervisory personnel concerned with bacteriological and chemical contamination of foods.

Emphasis will be given to methods, standards, and operating procedures applicable to a food sanitation program, including inspections, grading, and licensing of food establishments. Demonstrations and laboratory exercises include microbiological and chemical methods for food quality control, and procedures for enumeration, isolation, and identification of food poisoning bacteria.

Applications for both courses should be addressed to the Chief, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio, or to a USPHS Regional Office Director.

ADA Meets in Portland, Ore.

The American Dairy Association met in September at Portland, Ore., where N. S. GOLDING, Washington State College, reported on research work.

A new gravity balls test for determining the solids content of milk was demonstrated by Dr. Golding. Golding has worked for more than four years on a new approach which can be mechanized. He is anticipating the demand for a total solids test for milk to fit the needs of herd improvement testing as well as milk receiving. Golding reported that preparation of test sets is progressing in his laboratory. This rapid test will soon be ready for field testing in 10 to 15 colleges and university dairy laboratories.

The test unit uses a very simple combination of ten graded gravity test balls in a four ounce sample jar with a special polyethylene molded snap-in grid, through which the milk is added and the 17.6 ml. pipette inserted for the Babcock test. Further tests such as freezing point and gravimetric total solids can be made from the same sample.

The tests are made by liquefying the fat at 104°F., mixing, and adjusting the samples to the desired temperatures, remixing and reading the number of balls on the bottom of the jars placed on a glass plate. A mirror set under the glass plate provides for quick, easy reading, which can be checked by the number of floating balls. The submerged and floating balls total ten. Tests are then read quickly from special tables which involve the Babcock test and ball test. The grid keeps the balls in the jars during washing after each test and while the inverted jars are drying.

The American Dairy Association is continuing to support Golding's work for the fourth year, to have a number of experimental stations test the accuracy and practicability of the method under actual use conditions and against standard methods. All of this must be done before test units can be manufactured commercially.

Dairy science departments willing to organize a comparative testing plan in their herd testing work should contact A. O. SHAW or Golding at Washington State College, Pullman, Washington.

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Johnson & Johnson News

Eighteen prize-winning cows from all over the United States and Canada won Johnson & Johnson Rapid-Flo awards at the International Dairy Show held in Chicago in October.

The company's Filter Products Division gave cash prizes to first, second and third place winners in six, four-year-old classifications, Guernsey, Jersey, Brown Swiss, Milking Shorthorn, Ayrshire and Holstein.

Award winners included a grand champion, McDonald Farm's Jolly Token, a four-year-old Guernsey, owned by Cornell Univ. McDonald Farms, Cortland, N. Y. Another Johnson & Johnson prize winner was Mont's Tulip Dandy, which had already won the senior grand champion 4-H club award. The proud owner of Mont's Tulip Dandy is 15-year-old BEVERLY JOYCE MONTGOMERY, of Shelbyville, Tenn.

G. W. WILLITS, general manager of the Filter Products Division, said: "The Rapid-Flo Department makes these awards to help the dairy industry in its continued efforts to stimulate better breeding, increased output and quality milk production."

W. E. DORSETT has been named assistant general manager of Johnson & Johnson's Filter Products Division. Dorsett had been assistant to the vice president of sales for the medical and baby products manufacturer.

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Dorsett joined the company in 1946 as a general line salesman in Detroit. In 1950 he was assigned to the Chicago Division, where he had charge of the Milwaukee territory. Three years later he became manager of the Central Division, and moved to the home office and his general line sales post in 1956.

Dorsett, who is 35, attended Alma College, Alma, Mich. He also attended the Graduate School of Sales Management and Marketing at Rutgers Univ. During World War II he was a first lieutenant and pilot with the Eighth Air Force. He was awarded the Distinguished Flying Cross and the Air Medal with five Oak Leaf Clusters.

Completed Theses

Ph.D. Degree

M. J. FREUND—A study of the fructolytic activity of bovine semen. Rutgers Univ., New Brunswick, N. J.

H. D. LENNON—Plasma protein-bound iodine as a measure of thyroid function in dairy cattle. Rutgers Univ., New Brunswick, N. J.

M.S. Degree

R. R. ANDERSON—The clearance of phenolsylfonphthalein as a measure of renal function in dairy cattle. Rutgers Univ., New Brunswick, N. J.

N. NASHED BOSS—The comparative effects of initial fructose level and incubation temperature on fructolysis and percentage motile sperm of bull semen. Rutgers Univ., New Brunswick, N. J.

C. P. BREIDENSTEIN—Roughage with no grain and with medium and high grain feeding for milk production: grass-legume silage and pasture. Rutgers Univ., New Brunswick, N. J.

N. D. BROWN—The effects of various postpartum treatments upon the reproductive efficiency of dairy cows. Rutgers Univ., New Brunswick, N. J.

E. F. OGRINZ—Nutritive value of nitrogen-fertilized orchardgrass hay for milk production. Rutgers Univ., New Brunswick, N. J.

A. A. RIMM—Factors affecting roughage consumption in the dairy cow. Rutgers Univ., New Brunswick, N. J.

J. C. SIMMONS—The production, distribution and use of frozen, active lactic acid cultures. Clemson College, Clemson, S. C.

Borden's News

The creation of a Distribution Dept., headed by W. J. WILKES, to direct the physical distribution of its products, has been announced by T. O. HOFMAN, president of the Borden Foods Company.

The newly created department will combine much of the activities of the sales Service

Dept. and the General Traffic Dept. and will be responsible for the functional direction, research, cost study and control of the physical distribution of all products of the Borden Foods Company.

The Borden Foods Company was formed earlier this year by the merger of the former Borden Food Products and Borden Cheese Divisions. The new company has almost completed the establishment of a pattern of national sales districts, combining the sales forces of the merged divisions.

Mr. Wilkes will be charged with keeping pace with the ever changing distribution pattern in the food industry. He joined the Borden Company in 1922 and headed the Sales Service Dept. of the former Borden Food Products Division for the past six years.

The Borden Company has broken ground for the largest and most modern combined milk and ice cream plant ever built in Wisconsin. The $1,750,000 plant will rise on a 14 acre tract on Highway 100 in Milwaukee County, Milwaukee.

All Borden milk and ice cream operations in the Greater Milwaukee area will be served by the new plant. The one story building, with approximately 100,000 square feet of floor space, will combine the new separate operations of the Borden milk plant at 620 N. 8th Street and its ice cream plant at 1306 N. 4th Street.

A. F. TREBILCOCK, president of the Wisconsin Milk and Ice Cream Division of the Borden Company, announces that the combined plant operation will be under the supervision of D. K. BASKERVILLE, division general manager.

Two new instant food products will be introduced nationally in the near future by the Borden Company. The products are Instant Super Starlac nonfat dry milk, and Borden's Instant Whipped Potatoes. They are to be introduced on a market-by-market basis until full national distribution is achieved.

The Borden Company also announced the formal opening of its new Consumer Testing Laboratory and enlarged test kitchen, which are part of a stepped-up food research and development program.

The new Instant Super Starlac is not just an improvement on its predecessor, Instant Starlac, according to company officials. Its manufacture involves substantially different processing methods.

The Borden president described the new Instant Whipped Potatoes as a sharp departure from the Company's established product line, although evolving from a century of experience in dehydration and convenience foods. The product is the first of its kind to be offered to consumers in flake form. The flakes are freshly-mashed potatoes with the moisture removed, and the manufacturing process leaves the cellular structure of the potato undamaged.

Personnel at the company's $1,000,000 food research center at Syracuse, N.Y., has been increased by one half in the past six months and the technical staff had been increased by 75 percent. The Company will spend about 50 percent more for food research in 1958 than in 1957, and 1957 was its previous high.

The Test Kitchen and Consumer Testing Laboratory consists of a large room with ten private taste-test booths, five on each side. The two rows are separated by a serving aisle used by the home economists conducting the tests. A panelist cannot see the person serving, nor can he see the other panelists taking part in the test. Controlled, filtered lighting enables the economist to adjust the appearance of the product being tested. Special air conditioning removes any distracting odors. Sound-proofed ceilings and heavily carpeted floors screen out annoying sounds. Soft music is piped through loud-speakers in the ceiling to create a "cathedral-like" atmosphere encouraging concentration. During a test, each panelist completes a written questionnaire, scientifically prepared to bring out particular features or qualities of the product under study.

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NDC Speakers Announced

Seven prominent speakers to be featured at the National Dairy Council 44th Annual Meeting in Boston, Feb. 2-4, have been announced by MILTON HULT, NDC President.

They are Dr. PAUL DUDLEY WHITE, eminent heart specialist who attended President EISENHOWER; Dr. JAMES T. SHAW of the Harvard School of Dental Medicine; Dr. RALPH T. HOLMAN, Hormel Institute, Univ. of Minnesota, and Dr. F. BRUCE BALDWIN, Jr., Abbott's Dairies, Inc., Philadelphia.

The morning session will be concerned with subjects such as the value of nutrition research to public health, new research facts on dental health, providing special nutrition research information to the medical profession, and the role of NDC in nutrition research.

At the Tuesday luncheon the featured speaker will be Dr. SHANE MCCARTHY, Executive director of the President's Council on Youth Fitness.

Tuesday afternoon, in a session dealing with the values and needs of health education, EDWARD W. AITON, Director of 4-H Club and Young Men and Women Programs, Federal Extension Service, USDA, and GEORGE S. BULKLEY, The Carnation Co., Los Angeles and Chairman of the NDC Board, will deliver speeches and participate in a discussion session.

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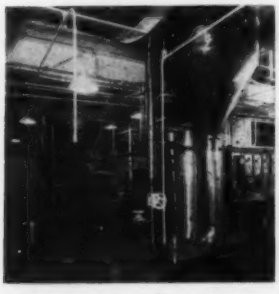

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Food Machinery, Chemical Corp. Promotes Pech

A. E. PECH has been named to the newly-created position of Manager—Milk Machinery Dept. of Food Machinery and Chemical Corp., San Jose, Calif.

A. E. Pech

In his new capacity, Mr. Pech assumes responsibility for contracts, machine specification and location in addition to his former responsibilities as technical service representative.

Mr. Pech has been engaged in dairy research and development work with the company for the past 17 years, having joined it shortly after completing his technical training at the Univ. of Wisconsin. During this period he held the position of chief of the Central Research Department's Dairy Products Section, and more recently, was appointed technical service representative of the Milk Machinery Dept.

Cherry-Burrell Corp. Acquires Samuel Olson Mfg. Co., Inc.

The Samuel Olson Manufacturing Company, Inc. of Chicago has been acquired by Cherry-Burrell Corporation of Cedar Rapids, Iowa, according to the announcement of HOWARD H. CHERRY, JR., president of Cherry-Burrell.

The Olson Company makes standard and customized equipment for materials handling. In recent years they have specialized in food conveying equipment for hospitals and for institutional cafeterias. They have also developed and installed equipment for conveying baggage at airports and for handling merchandise in mail-order houses and industrial plants.

The Olson firm will be operated as a wholly-owned subsidiary of Cherry-Burrell and its plant at 2418 West Bloomingdale in Chicago will continue producing its present line of conveyor equipment under the current management. PETER OLSON, president, will continue as head of the new subsidiary.

ABI News

Progress is being made in the consolidation of the offices of the American Butter Institute and the National Cheese Institute. Telephone number of both (ABI—NCI) *ANdover 3-2733-4.* Location—Room 620, 110 North Franklin Street, Chicago 6, Illinois.

A joint bulletin will be issued. The statistical information will be continued as previously.

The two organizations will retain their separate identities.

May, June '58 Journals Needed

Copies of the May and June, 1958, issues of the Journal of Dairy Science are urgently needed to take care of back copy orders, late subscribers, and late memberships.

If you can spare these journals, send them to the Journal of Dairy Science in care of The Garrard Press, 510 N. Hickory Street, Champaign, Illinois.

The Association will pay you $.50 for each copy you send plus actual shipping costs. As each shipment comes in, The Garrard Press will notify the Association of its contents, the sender, and the postage; the Association, in turn, will issue your check.

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SIGNIFICANCE OF STRONTIUM-90 IN MILK. A REVIEW

B. L. LARSON AND K. E. EBNER
Laboratory of Biochemistry, Department of Dairy Science, University of Illinois, Urbana

SUMMARY

Milk has received attention as a source of strontium-90 (Sr-90), since it is the major source of calcium and, hence, Sr-90, in the diet of most Western countries. Although several biologically active radioactive isotopes are produced in a uranium-235 atomic bomb explosion, Sr-90 is the major problem when considering long-term fallout, because of its slow fallout from the atmosphere, its long half-life of 28 yr., and its biological similarity to calcium. There are several discrimination steps against Sr-90 in its route to the bones of man after its deposition in soil from the atmosphere. These include the plant, the gut, the kidneys, the placenta, and the mammary gland. Compared to bone radiation received from other natural sources, the radiation from Sr-90 present in bone constitutes a small proportion, both now and if nuclear tests continue at the same rate. Milk contains much less Sr-90 per unit of calcium than do vegetables and cereals because of several discrimination factors in its formation, including the mammary gland. A comparison of diets consumed by various countries of the world indicates that those countries consuming a higher percentage of milk have lower relative Sr-90 intakes in their diets. Reliable data from which to draw sound conclusions are crucially lacking in many areas of the long-term fallout problem. The present knowledge strongly suggests that the current and projected levels of Sr-90 in milk should not cause us concern when compared to radiation received from natural sources; but further studies are necessary to be certain if this is true.

Although man has been concerned with the damaging effects of radiation for the past 30 yr., the threat of nuclear warfare and the current controversy over the testing of atomic devices have brought the problem to the immediate focus of public attention. The public has been made aware of the accumulation of radioactive materials from nuclear debris in its environment and of the possible damaging effects of this increase in radiation. In particular, the passage of strontium-90 (Sr-90) from the atmosphere into food and its accumulation in bone have been publicized.

It is recognized that milk is the principal source of calcium (and, hence, Sr-90) in the diet of most Western countries, including the United States. Various opinions on the significance of this fact have been advanced. One investigator *(3)* has proposed that all the calcium (and Sr-90) present in milk should be removed and replaced with calcium from uncontaminated "ancient sources"; conversely, it has been concluded that the levels of Sr-90 in milk are so low that we need not be concerned *(24)*.

The recognition of how much Sr-90 is present in milk and an interpretation and comparison of this level with that present in other food products, is of practical importance to the dairy industry. It is the opinion of the authors that there has been too much willingness on the part of the dairy industry to allow others not concerned with the industry to collect and to interpret the basic information relative to milk. Accusing fingers have been pointed at milk without proper

Received for publication August 13, 1958.

regard to the implications involved, with the result that the position of milk as an essential foodstuff, in the opinion of the American public, has been questioned.

The purpose of this review is to evaluate the available data on the accumulation of Sr-90 in milk and other foods and to discuss its significance. This review will be limited chiefly to the question of long-term radioactive fallout, since Sr-90 is not an important nuclide in the fallout resulting shortly after a nuclear explosion.

Background information. It is important for an understanding of the radioactive fallout problem to consider the primary nuclear reactions causing fallout, and to trace briefly the concern of scientists over the problem as it developed *(19i)*. In its early inception, the major problem was to win the war and little consideration was given to long-range fallout effects on behalf of the general population. In 1949, Dr. N. M. Smith of Oak Ridge undertook a theoretical analysis on the long-range aspects of fallout and, as a result, in 1952 Rand Corporation was given a special contract to make an independent study on the potential dangers of radioactive fallout. In the summer of 1953, at a conference called to review the Rand report, it was recommended that a study of mixed fission products be made, with emphasis being placed on Sr-90. As a result of this meeting, Project Sunshine was born, with Dr. W. F. Libby as its director. The immediate aim of this project was to analyze samples of soil, plants, animals, dairy products, human bone, and other materials from the United States and other parts of the world for levels of Sr-90. Originally, Project Sunshine was concerned with Sr-90 analyses, but now it is concerned generally with all phases of the fallout problem.

In late May and early June of 1957, the special Subcommittee on Radiation of the Joint Committee on Atomic Energy of the Congress of the United States, held investigations on the nature of radioactive fallout and its effects on man. Testimony and statements given at these hearings concerned all phases of the fallout problem and subsequently were published in a comprehensive 2,065-page report *(19)*. The release of this report made available the first major unclassified source of information relative to Sr-90 levels in milk, other foods, and forages. Unfortunately, some of the evidence and conclusions presented were undocumented with data; however, much has appeared in print since that time.

The units used to discuss radioactive phenomena are numerous and are apt to become confusing, since the terminology is not rigorous. The common terms used, as well as the specific term used to denote the concentration of Sr-90 in milk and other materials, are summarized in the Appendix.

Formation of Strontium-90. The hydrogen bomb uses a small atomic bomb to trigger the reaction. The atomic bomb is essentially a fission reaction in which uranium-235 (U-235) is bombarded by neutrons. A simplified scheme of the reaction may be represented by the following equation, where a neutron *(n)* bombards U-235, thereby releasing fission fragments *(f.f.)* and 2.5 neutrons, each containing two million electron volts *(19k)*.

$$n + \text{U-235} \rightarrow f.f. + 2.5n \text{ (2.0 Mev each)}$$

In the initial blast there are about 170 fission fragments produced, of which one is krypton-90, the radioactive precursor to Sr-90. The decay of Kr-90 to Sr-90 occurs by the successive elimination of beta (β) particles. The Sr-90 formed then decays further by eliminating β particles until stable zirconium-90 is formed.

$$_{36}Kr^{90} \xrightarrow[33\ \text{sec.}]{-\beta} {}_{37}Rb^{90} \xrightarrow[2.7\ \text{min.}]{-\beta} {}_{38}Sr^{90}$$

krypton — rubidium — strontium

$$_{38}Sr^{90} \xrightarrow[28\ \text{yr.}]{-\beta} {}_{39}Y^{90} \xrightarrow[65\ \text{hr.}]{-\beta} {}_{40}Zr^{90}$$

strontium — yttrium — zirconium

The time indicated below each arrow is the half-life of that element and represents the time for one-half of a given quantity of the element to decay. The long half-life of 28 yr. for Sr-90 is one of the properties that make it a problem in long-term fallout. Y-90, the daughter of Sr-90, eliminates a β particle prior to forming stable Zr-90. Its half-life is only 65 hr.; thus, the radioactive effects of Sr-90 are actually due to the combined disintegrations of Sr-90 and Y-90 to stable Zr-90 *(1, 19k)*.

There are four natural isotopes of strontium, Sr-84, Sr-86, Sr-87, and Sr-88, all of which are nonradioactive *(19o)*. In the fission reaction six isotopes of strontium are produced, all of which are radioactive. Three of these have extremely short half-lives and are of no practical concern. The other three, Sr-91, Sr-90, and Sr-89, all decay to Y-90 and, finally, to Zr-90 which is stable. Sr-91 has a half-life of 10 hr. and Sr-89 has a half-life of 53 days. These two are of little concern in studies on long-term fallout because of their short half-life, and their decay product, Y-90, is not absorbed readily by the digestive tract.

Other principal isotopes of biological interest produced in the fission reaction are summarized in Table 1, and their occurrence as a function of time after the initial blast is given *(19m)*. The radioactive isotopes of iodine can be concentrated readily by the thyroid, but are of little importance in long-term fallout, since their half-lives are very short. The half-life of I^{131} is eight days. The rare

TABLE 1

Principal biological isotopes from slow neutron fission of 1 kg. of U-235 (19m)

Time after fission	Isotope	% Total activity
1 day	I-133	7.3
	Sr-91	6.7
	Ce-143	6.7
	I-135	5.7
	I-131	0.9
20 days	Ba-140	12.0
	Ce-141	9.7
	I-131	5.6
	Sr-89	5.0
	Ce-144, Pr-144	2.6
1 yr.	Ce-144, Pr-144	52.8
	Sr-89	2.7
	Sr-90, Y-90	3.7
20 yr.	Sr-90, Y-90	48.0
	Cs-137, Ba-137	45.0

earths, such as cerium^{144} and praseodymium, are not absorbed from the gut and also are very poorly taken up by plants. Barium^{140} is not a problem, since its half-life is only 13 days.

Cesium^{137} (Cs^{137}) is related chemically to potassium and is distributed in the body somewhat like potassium *(1, 19a)*. That is, it is found in muscle tissue, blood, and other soft tissues. Cs^{137} has a half-life of 27 yr., while its daughter, Ba^{137}, has a half-life of 2.6 min. Because of its long half-life, Cs^{137} would be a potential danger to the body, but fortunately it is removed fairly rapidly. The biological half-life in the body is 200 days. Another factor lessening the absorption of Cs^{137} in man is that plants take up little cesium from the soil colloidal complex.

The pathway of Strontium-90 into man. Sr-90 is potentially the most dangerous nuclide to man that is formed in the fission reaction. Studies on the geographical distribution of the Sr-90 blown into the atmosphere indicate that the majority has remained in the temperate zone of the northern hemisphere *(10,19e)*. Sr-90 has a long physical and biological half-life and is produced in relatively high yields in the fission reaction. The Sr-90 formed probably combines with oxygen to form $Sr^{90}O_2$, which gradually settles onto the earth's surface. It has been estimated that it takes 7–10 yr. to settle out one-half of the amount of Sr-90 formed from a blast *(10, 14)*.

Chemically, strontium and calcium are similar, since they are in the same group of the periodic table, but physically they differ, since strontium is about two and one-half times as heavy as calcium. There are several discrimination steps against the uptake of Sr-90 in its passage from the atmosphere to the bones of man and, apparently, this is due to the differences in physical properties of the two elements.

The Sr-90 deposited on the earth either remains on vegetation which may be eaten by animals or is washed into the soil. That which is washed into the soil is bound to the colloid complex similarly to calcium and is retained in the top few inches, thus being readily available to short-rooted plants. Plants discriminate against Sr-90 by absorbing it about 0.7 times as fast as calcium if sufficient calcium is available *(5, 15)*. When plants are eaten by animals, a portion of the Sr-90 absorbed is deposited rather permanently along with calcium in the bone, though at a somewhat slower rate than calcium. This deposition occurs mainly at sites of active bone growth and bone tissue replacement. Sr-90 can also be passed from the blood stream into a developing fetus and into milk.

Since Sr-90 and calcium metabolism are closely associated, concentrations of Sr-90 are generally expressed in terms of calcium. The most common unit used is the Sunshine Unit (S.U.), which is equal to the number of micromicrocuries of Sr-90 per gram of calcium (see Appendix). It is important to note that the Sunshine Unit represents the ratio of Sr-90 to calcium present. In itself it is not an absolute amount of Sr-90, although it is often used in this sense by assuming that the calcium level in the body is 1,000 g.

Estimated values of the discrimination against Sr-90 in the passage from

plant to man vary, according to the investigator. Most authorities estimate that the discrimination factor for cow's milk is about 0.09 to 0.14; that is, that there are seven to 11 times more Sunshine Units present in the cow's ration than appear in the milk *(5, 8, 15, 19o)* (see Appendix for Discrimination Ratio definition). Studies in rats and rabbits indicate a discrimination ratio of about 0.5 between the mother's blood and the fetus *(5, 19l)*. The rato of Sr-90 to calcium present in the milk of lactating goats was found to be 0.38 of that present in the blood *(19a, 22)*. It should be noted that discrimination factors or ratios may be expressed either as whole numbers or as their reciprocal fractions.

In the animal, the principal sites of discrimination are the gut, kidney, placenta, and mammary gland. Neuman *(19l)* has schematically summarized the discrimination scheme in the body and uses a factor of two for each site. This scheme is shown in Figure 1. The discrimination by the gut is apparently

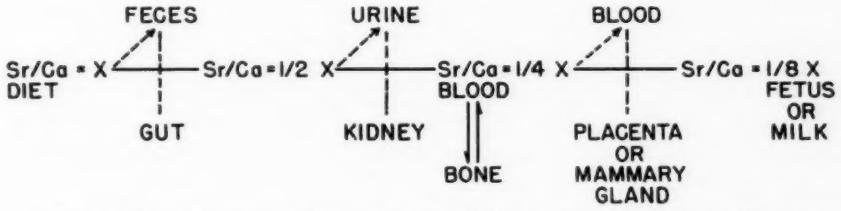

FIG. 1. Schematic summarization of major discrimination sites against Sr-90. A discrimination factor of two is assumed for each site *(19l)*.

influenced somewhat by the diet. It has been reported that if the diet is primarily milk the discrimination by the gut is lowered *(5, 19l)*. The discrimination ratio from diet to bone shows considerable variation, but the more recent work indicates a value of four *(8, 15)*.

These discrimination values indicate that the Sr-90 content of milk per gram of calcium should be less than vegetables or cereals, because of several discrimination sites, and also less than other animal foodstuffs because of the additional discrimination by the mammary gland. However, the possibility that human and animal foods differ in their Sr-90 content and that the human gut does not discriminate as well against Sr-90 on a diet high in milk must also be considered in any evaluation. This will be considered more fully later.

The levels of Sr-90 in milk. Apparently, it has been only since April of 1954 that milk has been routinely analyzed for its Sr-90 content. These analyses are under the supervision of the Atomic Energy Commission and are a part of Project Sunshine. One of the aims of Project Sunshine was to find an index by which the Sr-90 levels in human bone could be readily estimated. Milk and its products were a natural choice, since in the United States the majority of the dietary calcium comes from milk products and, thus, the levels in milk should parallel those found in human bone. If the discrimination factor from milk to bone and the Sr-90 level in milk is known, the Sr-90 content in human bone can be readily estimated. Also, the monitoring of milk is facilitated by the fact

that fairly representative samples are easy to obtain from different parts of the United States and the world. It is chiefly for these reasons that the general public has become more aware of Sr-90 in milk than in other food products.

Procedures for analyzing Sr-90 are generally long and tedious and are subject to considerable variation. The common method used is to ash the sample, dissolve it in hydrochloric acid and add a carrier of radioactive yttrium, which is subsequently precipitated as the oxalate and counted in special low-level counters *(23)*. Since there is some experimental error, the values reported may not be absolute and some caution must be used in interpreting data. The present level of Sr-90 in milk which gives only 10–15 disintegrations per quart per minute *(23)* illustrates the difficulties in making such low-level counts.

Since April of 1954 in the United States, monthly analyses of Sr-90 in milk samples from six major milk sheds have been made for periods ranging from

TABLE 2

Sr-90 content of dried milks (19e)

Date	Location							
	Perry, New York	State College, Miss.	St. Louis, Mo.	Columbus, Wis.	Mandan, North Dakota	Portland, Oregon	Japan	United Kingdom
	(Sunshine units)							
1954—April	0.47	.....	.....	.....	.....	.....	.....	.....
May	1.2	.....	.....	.....	.....	.....	.....	.....
June	1.3	.....	.....	.....	.....	.....	.....	.....
July	1.5	.....	.....	.....	.....	.....	.....	.....
August	1.2	.....	.....	.....	.....	.....	.....	.....
Sept.	1.5	.....	.....	.....	.....	.....	.....	.....
Oct.	1.4	.....	.....	.....	.....	.....	.....	.....
Nov.	1.1	.....	.....	.....	.....	.....	.....	.....
Dec.	0.64	.....	.....	.....	.....	.....	.....	.....
1955—Jan.	2.5	.....	.....	.....	.....	.....	3.0	.....
Feb.	0.77	.....	.....	.....	.....	.....	1.0	.....
March	0.75	.....	.....	.....	.....	.....	2.0	1.8
April	0.31	.....	.....	.....	.....	.....	1.8	2.9
May	1.9	2.6	4.1	1.0	7.3	1.7	1.9	.....
June	2.5	4.7	4.6	4.6	9.2	2.6	0.8	5.5
July	1.9	4.4	3.9	0.8	6.3	.....	.....	2.6
August	2.0	4.1	.....	1.2	5.8	.....	0.8	.....
Sept.	1.5	3.2	.....	3.3	4.7	.....	2.0	.....
Oct.	2.8	.....	.....	4.4	6.9	.....	7.5	.....
Nov.	2.5	.....	.....	3.7	7.4	.....	2.5	.....
Dec.	3.3	.....	.....	3.0	10.0	.....	3.5	.....
1956—Jan.	2.3	.....	.....	3.0	3.5	.....	2.7	4.0
Feb.	2.0	.....	.....	3.5	8.1	.....	.....	.....
March	2.0	6.3	.....	3.4	11.0	.....	3.5	.....
April	2.9	6.7	.....	3.4	9.6	5.2	3.0	4.6
May	2.8	4.9	.....	2.8	17.0	6.4	.....	4.5
June	3.0	4.4	.....	3.4	8.7	5.0	.....	5.0
July	2.7	6.1	.....	4.2	6.6	.....	2.3	.....
August	3.1	3.8	.....	4.7	8.6	.....	.....	.....
Sept.	4.9	4.8	.....	4.3	10.7	.....	2.7	.....
Oct.	5.4	.....	.....	4.72	8.9	.....	.....	.....
Nov.	5.6	.....	.....	.....	3.6	.....	.....	.....
Dec.	.....	.....	.....	.....	.....	.....	.....	.....
1957—Late *(11)*	4.5	.....	6.5	5.5	10.0	7.0	.....	.....

1 to 4 yr. These analyses were made on dried milk samples that were obtained at local plants. In January of 1955, intermittent analyses were carried out for Sr-90 in milk obtained from Japan and the United Kingdom (primarily England). The results of these analyses until December, 1956, are presented (Table 2) and are expressed in Sunshine Units *(19e)*. The most recent summary of the average Sr-90 content of dried milk from various parts of the world as of late 1957 are also tabulated in Table 2 *(11)*.

These data show that there has been an increase in the Sr-90 content of milk in different parts of the world. The 1956 values (Table 2) for Japan appear low when compared to the previous values in the table, but this is probably due to the fact that only a few samples were analyzed in 1956. The amount of Sr-90 in dried milk for Mandan, North Dakota, is high in comparison with other areas of the United States and is apparently due to the deficient levels of calcium in the soil of that region. As a result, the deposited Sr-90 is absorbed more efficiently by plants.

There are few data available on the Sr-90 content of fresh milk, though a study has been carried out in the Chicago area *(19j)*. Samples of fresh milk were obtained monthly from several large Chicago dairies from March, 1955, to March of 1956. The milk samples were analyzed for Sr-90 and the results are presented (Figure 2). The curve in Figure 2 shows a maximum from June to

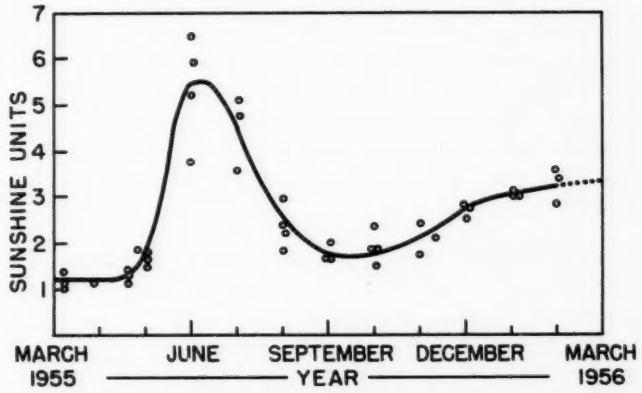

FIG. 2. Monthly fluctuations in Sr-90 content of fresh milk in Chicago *(19j)*.

August in 1955, followed by a minimum and then a gradual rise to about 3 S.U. at the end of 1956. This is in agreement with the Columbus, Wisconsin, value of 3.4 S.U. at the end of 1956. The peak in the curve may be attributed to the fact that there was a heavy series of tests at Nevada that spring and when the cows were pastured on unplowed fields they received an increased level of Sr-90 from surface contamination of the plants. The level of 1–2 S.U. in March and April of 1955 was apparently due to the fact that the cows were eating stored feed which had been grown the previous summer.

Comparison of Sr-90 levels in milk with other foods. There has been approximately a fourfold increase in the average world Sr-90 content of milk from 1954 to the level of 5–6 S.U. in 1958. Unfortunately, the present data on the Sr-90 content of other foods are limited, but they are gradually being collected through the efforts of Project Sunshine and other agencies such as the U. S. Public Health Service. Fish have an extremely low Sr-90 level because of the low concentration of Sr-90 in the oceans, caused by dilution. Meat and eggs (not including the shells) contain little calcium and thus little Sr-90. Vegetables contain Sr-90 and an average value in Sunshine Units for various market frozen vegetables in 1956 was about twice that found in milk *(8, 11)*. Data on cereals also are meager, but apparently their levels of Sr-90 in Sunshine Units average about two and one-half times that of milk. In general, the level of Sr-90 in vegetables and cereals consumed by humans would be expected to be lower than the levels found in the same materials consumed by animals. Human foods are generally processed and surface contamination is largely removed. Furthermore, vegetables and, to some extent, cereals for human consumption are usually grown on well-fertilized, carefully cultivated soils; whereas, animals generally consume forage grown on less carefully maintained soils.

The 1957 level of about 5 S.U. of Sr-90 in milk suggests that the average level of Sr-90 in the ration of the dairy cow was about 40–50 S.U., because of the apparent discrimination factor of 7-11 *(19h)*. The average cow producing market milk in the United States receives supplements of rock calcium phosphates containing a low level of Sr-90 and/or animal bone meal containing about 5–10 S.U. Since this is discriminated against by a factor of 7–10 in passing to the milk, it is apparent that the present level of Sr-90 contributed by the succulent plants, grain, cereals, etc., consumed by the cow must be quite high. Data on this point are limited and show wide variations. Levels of Sr-90 found in cereals, vegetables, other plant sources, and water are shown in Table 3. The

TABLE 3

Sr-90 content of various human and animal plant sources

Source	Reference	Year	Location	Sr-90 Level	
				Range	Average
				—*(Sunshine units)*—	
Vegetables (frozen)	*(8)*	1956	U.S.	1–21	7
Vegetables (frozen)	*(11)*	1957	U.S.	1–29	9
Wheat	*(11)*	1956	U.S.	9–38	23
Oatmeal	*(11)*	1956	(?)		6
Rice	*(17)*	1956	Japan	81–250	150
Rice	*(11)*	1956	(?)		4
Flour	*(11)*	1956	U.S. (Ill.)		6
Bran	*(11)*	1957	U.S. (Mich.)		9
Bean plant (parts)	*(19f)*	1956–7	U.S. (Md.)	4–81	45
Pea plant (parts)	*(19f)*	1956–7	U.S. (Md.)	19–39	30
Alfalfa	*(19j)*	1955	U.S. (Ill.)	3–38	18
Grass (normal soil)	*(19b)*	1956	England	23–85	40
Grass (acid soil)	*(19b)*	1956	England	113–2,180	370
Hay	*(19c)*	1955	England	6–53	30
Drinking water	*(11)*	1956–7	U.S. (N.Y.)		0.1[a]

[a] About 0.1 $\mu\mu$c. of Sr-90 per liter. Based on consumption with 1 g. of Ca in diet per day.

data suggest that plant foods prepared for human consumption do contain a lower Sr-90 content than do plants consumed by animals.

From the incomplete data available, it is difficult to assess average values for the levels of Sr-90 present in various foods consumed by humans, though one can make some guesses on relative levels. Kulp and Slakter *(11)* have evaluated the Sr-90 intake of humans in the United States in late 1957, using as a standard a level of 6 S.U. in milk, 10 S.U. in vegetables, and 15 S.U. in cereals. They calculated an average Sr-90 intake of 6.5 S.U. for a person in the United States. Using these values, and knowing the source of dietary calcium, it is then possible to estimate the average Sr-90 intake of people in the various countries of the world. Any such estimation suffers, since specific data on the level of Sr-90 in the food in each country are not available and the effect of the total calcium level in the diet has not been well-defined *(18)*. However, such a calculation does show the effect a shift in eating habits could have on the relative Sr-90 intake of a population.

The various countries of the world, because of their differences in diet, receive a different percentage of their dietary calcium from cereals, vegetables, milk, and other minor products such as fish, meat, eggs, etc. The sources of calcium in national diets for a few representative countries are tabulated in Table 4 *(8)*.

TABLE 4

Sources of calcium in national diets (8)

Country	Cereals	Vegetables	Meats, eggs, fish	Milk products
	(% total calcium intake)			
Sweden	4	6	4	87
United States	4	5	7	85
France	8	12	5	75
Italy	19	14	5	62
Peru	17	37	5	41
Portugal	26	35	9	30
Japan	46	25	11	18

Twenty-one of the 35 countries listed by Eckelman *et al. (8)* receive more than 70% of their dietary calcium from milk products. The countries selected in Table 4 illustrate the large differences in the source of dietary calcium. If the following Sr-90 levels are used, based on milk as 6 S.U., vegetables as 10 S.U., and cereals as 15 S.U., and the small contribution from other foods is ignored, then the relative total amount of Sr-90 received in the diet, as well as the per-

TABLE 5

Estimated source of dietary Sr-90 for various countries

Country	From cereals	From vegetables	From milk products	Sr-90 in total diet
	(% total Sr-90 intake)			*(Sunshine units)*
United States	9.7	8.1	82.2	6.2
Italy	35.8	17.6	46.6	8.0
Portugal	42.4	38.0	19.6	9.2
Japan	65.9	23.8	10.3	10.5 (73)[a]

[a] On basis of 1956 rice crop *(47)*.

centage from each source, can be calculated. Results of this calculation are tabulated (Table 5).

The countries chosen for the calculations in Table 5 have greatly different sources of dietary calcium. For example, people in the United States receive 82% of their dietary Sr-90 from milk products but, in fact, have the lowest level of Sr-90 in Sunshine Units in their diet. The two extremes are the United States and Japan. Only 10% of the calcium in the Japanese diet comes from milk products. On the basis of this calculation, the total intake of Sr-90 for Japan is about two times that of the United States, because of the high level of cereals consumed and, in fact, may be many times higher, since the cereal consumed is largely rice, which has a short-root system *(17)*.

It is apparent that if, by some means of adverse advertising and ignorance on the part of the public, the calcium diet of the United States were shifted away from milk to cereal or vegetable products the population would be increasing the relative level of Sr-90 in their diet.

Influence of a milk diet on discrimination. The reason why a diet high in milk products contains less Sr-90 than other foods is because of the additional discrimination factors operating in its formation. The data in Table 6 represent dietary Sr-90 and not that finally deposited in the bone. There is evidence to indicate that as the ratio of milk in the diet increases the discrimination against Sr-90 in the passage across the gut decreases *(5, 6, 7, 12, 20, 21)*. It is known that certain materials increase calcium absorption, and milk is noteworthy in this regard. Comar *et al. (5, 12, 20)* have shown that specific substances, such as lactose, lysine, and arginine, which tend to increase calcium absorption, are even more effective in increasing strontium absorption. Thus, the normal discrimination from the diet to bone is about four, but on a milk diet may approach two *(5, 6, 7, 12)*. Rather conclusive evidence on this point is available for the rat, although the proportion of milk in the diet of these studies was much higher than would be present in a normal human's diet. The data concerning humans are not so convincing. Such studies generally have involved Sr-89, and a limited number of individuals who were hospitalized with advanced serious carcinomas; all of these subjects did not show less discrimination against strontium on the milk diet.

Kulp *et al. (8, 11)* use the discrimination factor of four between diet and bone for individuals in the United States. This value is based on the actual observed difference between the average level of Sr-90 in the diet of persons of varying ages in the United States and the actual level found in bone *(8)*. Again, these data are limited, because of the lack of data on normal diets. The reasons for a discrepancy between the limited laboratory experiments of Comar *et al. (5, 6, 7, 12)* and the actual observed values of Kulp *et al. (8, 11)* are not known. It may be that such data from rats can not be extrapolated to humans and that the limited studies with humans are in error. It is more likely, however, that the form in which milk and milk products are consumed regulates the discrimination factor. Lactose was the most powerful calcium and strontium absorption agent found by Comar *et al. (5, 12, 20)*; yet, many dairy products consumed by humans,

TABLE 6

Radiation received by humans from various sources

Source	Milliroentgens per yr.	Equivalent sunshine units	Reference
External radiation:			
Background—granitic rock			
Sea level	147		*(13)*
5,000 ft.	170		*(13)*
10,000 ft.	230		*(13)*
Background—sedimentary rock			
Sea level	80		*(13)*
5,000 ft.	103		*(13)*
10,000 ft.	163		*(13)*
Background—open oceans	57		*(13)*
Wrist watch	10–40		*(13, 19g)*
X-ray (routine chest)	500–2,000		*(19g)*
X-ray (anterior-posterior body)	3,000		*(13)*
X-ray (lateral body)	7,000		*(13)*
Brick house	25–50		*(14)*
Earth fallout from nuclear detonations in 1956	1–5		*(14, 19g)*
Soft tissue internal radiation:[a]			
Potassium-40	19		*(13)*
Radium	2		*(19g)*
Carbon-14	1.5		*(13)*
Cesium-137	1		*(19g)*
Equivalent internal bone radiation:[b]			
Radium	6.7, 120	2.2, 40	*(13, 14)*
Potassium-40	5	1.7	*(19g)*
Cosmic rays—Sea level	37	12	*(14)*
5,000 ft.	60	20	*(14)*
10,000 ft.	112	37	*(19g)*
Strontium-90[c]			
Now (1957-8):			
Children	1.5	0.5	*(14)*
Adults	0.6	0.2	*(11)*
In 10 yr. if testing stops now:			
Children	4.8	1.6	*(11)*
Adults	1.2	0.4	*(11)*
If testing continues:[d]	12–63	4–21	*(11, 14)*

[a] In addition to external radiation, much of which penetrates tissue.

[b] External radiation (chiefly γ) other than cosmic rays also is active at the bone level. It is difficult to assess the proportion that actually is. Dudley and Evans *(19d)* assume that about 70% of the radiation from the ground rocks is effective at the skeletal level.

[c] Eckelman *et al. (8)* estimate that the standard deviation for Sr-90 in individual persons will be about 40% of the mean at equilibrium.

[d] Value at 50 yr. if testing continued at same rate with same type devices as past 5 yr. New Sr-90 entering atmosphere is in equilibrium with that falling out.

such as cheese, cultured milks, etc., contain a negligible amount of lactose. Furthermore, milk and milk products are generally consumed in conjunction with other foods, where the effect may be minimized.

The immediate need for further investigations in this area is apparent. Conclusive data are needed concerning the discrimination against Sr-90 in humans on a milk diet and concerning the effect of specific dairy products. If it is true that certain dairy products, or the way in which they are consumed, do not allow the gut to discriminate as well against Sr-90, then it would be preferable if the dairy industry itself would find out the facts about them. It would be undesirable to let someone else not directly concerned with the industry throw a

cloud of suspicion over certain dairy products, with opinions based on incomplete studies. At the very least, the conflicting data relative to the average discrimination against Sr-90 in the whole population on a milk-containing diet need to be resolved immediately.

Danger of radioactivity in foods. The question of the extent to which ionizing radiations are harmful to man has received considerable attention by many investigators and as to date the results are generally inconclusive *(2, 9, 14, 16, 19g)*. Some believe that all radiation, regardless of dosage, is harmful and cumulative; others believe that a threshold level must be reached at any given time before there is any biological damage. Biological damage can be caused by high levels of radiation and it is postulated that the ionization caused by radiation leads to the formation of powerful oxidizing radicals. One of the most important consequences of this is enzyme and other protein alteration, either by oxidation of sulfhydryl groups or protein denaturation. The effect of ionization on ribo- and desoxy-ribonucleic acids is shown by a reduction in nucleic acid synthesis and a reduction in total but an increase in abnormal mitoses depending on the dosage received.

The debate between various authorities over the effects of low-level radiation arises because measurements are difficult to make. The effects of large doses of radiation are most apparent and they affect first the hematopoietic or blood cell-forming system. The germ cells also are quite sensitive, as are the cells of the intestinal mucosa. Bone, liver, and brain cells are less affected by large doses of radiation. It is postulated that long-term, low-level radiation may cause increased tumors of many types, including bone cancer, leukemia, and shortening of the life span.

Investigations with small animals have indicated that their life span is shortened when exposed to high levels of radiation. If such data are extrapolated to humans (there is no direct evidence that such an extrapolation is valid), then the presently accepted life-time maximum dose from all sources of 100 roentgens (r) over a period of 70 yr. would reduce man's life span by 500 days *(19g)*. Estimates have also been made by life insurance companies for the reduction of a 70-year life span by various natural habits: for example, 25% overweight, −3.6 yr.; 1 package of cigarettes per day, −7.0 yr.; driving a car in the United States, −400 days *(19g)*. If these data are valid, then the present accepted life-time dose would not reduce life span any more than accepted practices.

Humans receive radiation from many sources besides Sr-90. Cosmic rays, radium present in earth, bones, wrist watches, etc., potassium^{40} and cesium^{137} present in earth, foods, and tissues, x-rays, and other sources all contribute to the total dosage received. Such radiation sources are listed (Table 6). External radiation amounts to about 150 milliroentgens (mr) per year, with wide variations in the population. Altitude and radiation from the ground account for some of the largest variations. A dosage of 150 mr per year is equivalent to about 10 r of radiation in a 70-yr. life span, or 10% of the present accepted maximum life-time dosage.

In making comparisons of radiation sources, it must be taken into account that Sr-90 radiation is localized in the bone and all of the other sources of external radiation can not be compared directly because of differences in penetrating power. However, Libby *(14)* has pointed out that it is possible to compare directly Sr-90 and cosmic ray radiation at the bone level. The β-particles eliminated by Sr-90 and Y-90 decomposition have a tissue penetration of about 2 mm. and this is localized in the bone where cell formation is taking place. On the other hand, cosmic rays penetrate the whole body, and the ionization density along the tracks of the μ-mesons, which are the principal cosmic ray components to consider in the lower air, is similar to that of the Sr-90 and Y-90 decomposition. Hence, it is possible to equate cosmic-ray dosage and Sr-90 dosage directly, and express cosmic ray radiation in terms of Sunshine Units. Libby *(14)* notes that the annual cosmic-ray radiation dosage at Washington, D. C. (or any place at sea level at this latitude) is about 37 mr per year and at Denver, Colorado (5,000 ft.), 60 mr per year. This is equal to about 23 mr per year difference, or is equivalent to 8 S.U. (1 S.U. is about the same as 3 mr per year). The present level of Sr-90 in the bones of children is between 0.5 and 1.0 S.U. and, if testing were to continue at the same rate indefinitely to equilibrium, the level of Sr-90 in human bone would be in the range of 4–21 S.U. *(8, 11, 14)*. This level compared to 8 S.U. derived from cosmic rays at Denver over Washington, suggests that if the level were strong enough to cause leukemia and bone cancer there should be a correlation of such diseases with altitude. Examination by Libby of Public Health records showed that this is not the case *(14)*.

It is apparent that the present levels of Sr-90 in bone are contributing a small amount of radiation compared to other sources. If testing were to continue for the next 50 yr. at the same rate, the contribution from Sr-90 in bones would be about equivalent to moving from sea level to 5,000 ft. altitude.

At present, the maximum permissible dose of Sr-90 in bone is 100 S.U. for the average population. The present average level in children is less than 1% of this and will rise only to 4–20% of this, at the extreme, if testing continues. One should also not underestimate the abilities of the atomic scientists. Since it is the U-235 of the atomic bomb detonator which produces Sr-90, and not the hydrogen bomb itself, the development of a "clean" means of starting the hydrogen bomb would lower or eliminate Sr-90 formation. The radiation produced from a hydrogen bomb is chiefly due to tritium, which is an extremely weak β particle emitter. Attempts are now being made to develop such a system.

Genetic effects of radiation. While this review has been concerned chiefly with the significance of Sr-90 in milk, it is unwise to neglect the genetic considerations of the fallout problem. This is necessary because there is not a clear division point between the various phases of the fallout problem. As a source of radiation to the gonads, the Sr-90 derived from food and present in bone is of small consequence because of the low level present and the short penetrating power of the β particles. However, the fallout of other isotopes produced in the fission reaction has increased the background radiation. Although this increase

is quite small (see Table 6), at present the greatest debate over the continuation of nuclear tests centers around the genetic effects this increased radiation may have on future generations. These effects are extremely difficult to evaluate since so little is known about human genetics. Judging from experience with plants, insects, animals, and other lower organisms, there is every reason to expect some genetic effects of radiation. It seems likely that a major portion of the spontaneous mutations of the human species is not due to radiation but to other causes. Muller [quoted by Libby *(14)*] has estimated that 10% of the spontaneous mutations in the human species may be due to irradiation. If one estimates that the 150 (mr) per year from natural radiation now causes 10% of the spontaneous mutations, then the test fallout, if continued indefinitely at the present level would cause an increase in the over-all natural spontaneous mutation rate of about 0.2% *(14)*. This effect is comparable to moving to a slightly different locality and is much less serious than changing houses or doing any one of a dozen different things *(14)*.

At present in the United States some 80,000, or 2%, of the four million children born alive each year have some tangible genetic defects *(2)*. The increase expected, if estimates and extrapolations to low levels are justified and the tests continue, would be about 160 children per year at the 0.2% level *(2)*. Whether this effect can be considered small compared to unavoidable damage caused by spontaneous mutations and other presently accepted hazards of life depends on ethical and emotional differences in individuals. We as a country accept death and maiming through preventable accidents such as those from jobs, sports, automobiles, etc. We discount the harm by considering the advantages. Also, the individual believes he can exercise some control over them. The fallout hazards are beyond the control of the individual, involve his descendants, and so have a strong emotional impact. The need for further controlled studies in this area is apparent. From the evidence at hand, one must conclude that the present and projected increases in radiation due to nuclear detonations, if testing is continued at the same rate, are small enough so that they should not cause us undue concern until further facts concerning humans are available.

APPENDIX

Common Radioactive Terms

1. *Curie (c):* The absolute unit of radioactivity equal to 3.7×10^{10} disintegrations per second. The microcurie (μc) is equal to 3.7×10^{4} dps. *(4)*.
2. *α-Particles:* Nuclei of helium atoms with high ionization power but low penetrating power.
   β-Particles: Electrons traveling at high velocities with better penetrating but less ionization power than α-rays.
   γ-Rays: Light or x-rays with high penetrating power and comparable biologically to β-rays in ionization power.
3. *Roentgen (r):* A quantity of radiation such that the associated corpuscular emission per 0.001293 g. of air produces, in air, ions carrying one electrostatic

unit of quantity of electricity of either sign. The roentgen is a measure of energy absorbed and corresponds to the absorption of 83 ergs/g of air and about 93 ergs/g of tissue *(4)*.

4. *rep:* Physical equivalent of a roentgen and equals 93 ergs/g *(4)*.
5. *rad:* A unit of absorbed dose which is equal to 100 ergs/g *(4)*.
6. *RBE:* Relative Biological Effectiveness. RBE compares the biological effectiveness of radiation from different sources. For β particles and γ rays, RBE = 1; for α particles, RBE = 20, and for protons, RBE = 10. This unit is not precise, for it depends on pH, oxygen tension, and the organ radiated *(19k, 19n)*.
7. *rem:* It is the unit of RBE dose. That is, it includes the RBE and the absorbed dose in rads. This unit also has a degree of uncertainty *(19n)*.
8. *Discrimination ratio:* The ratio of strontium to calcium in any product compartment decided by the ratio in a precursor compartment. Comar *et al. (5)* express it as the "Strontium-Calcium Observed Ratio": $OR_{\text{sample-precursor}} = \frac{\text{Sr/Ca of sample}}{\text{Sr/Ca of precursor}}$. For example, an $OR_{\text{milk-diet}}$ of 0.1 means that the diet of a cow contains ten times as much Sr-90 per unit of calcium as is found in the milk. This may also be expressed as a Discrimination Ratio, or Factor of 10.
9. *Sunshine Unit (S.U.):* A S.U. is equal to the number of micromicrocuries of strontium-90 per gram of calcium ($\mu\mu$c Sr-90/g Ca) and is the common unit for describing the concentration of Sr-90 in man *(19n)*. It is equal to one-hundredth of the average permissible body burden of Sr-90 for the general public (0.1μc). Since the body contains about 1,000 g. of Ca, the maximum permissible amount of Sr-90/g Ca for the average population is equal to 1×10^{-4} μc Sr-90/g Ca. The Sunshine Unit is 1/100th of this value, or equal to $\mu\mu$c Sr-90/g Ca.

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HETEROGENEITY OF CRYSTALLINE RENNIN[1, 2]

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SUMMARY

Block-shaped crystals were prepared from a concentrated solution of purified rennin by dialyzing it against Berridge's salting-in buffer. The purification procedure was relatively simple, and proved successful in each of several attempts to obtain rennin pure enough to crystallize. A single boundary in electrophoresis represented over 96% of the protein when the dissolved crystals were run at pH 6.8 in sodium phosphate buffer at an ionic strength of 0.2. However, at least four components were evident in the same buffer at 0.033 ionic strength. These results show that rennin preparations can not be considered homogeneous on the basis of their crystalline form or their electrophoretic behavior in phosphate buffers of high ionic strength.

Several European workers *(1, 4, 10, 13)* have crystallized rennin in the form of rectangular plates or cubes. Electrophoretic examination of the crystals dissolved in 0.1 *M* phosphate buffer led Alais *(1)* to conclude that his preparation was electrophoretically homogeneous. Schwander *et al. (25)* had found earlier that Berridge's *(5)* crystalline rennin behaved uniformly in paper electrophoresis, but showed some evidence of heterogeneity in sedimentation and diffusion experiments. Berridge *(8)* felt that this heterogeneity resulted from using an old sample of crystals which might have sustained some decomposition, or that the enzyme was partially inactivated while running the experiments at pH 7.0. By use of the constant solvent solubility test *(11)*, he had shown earlier that, under one set of conditions, rennin activity was proportional to nitrogen in solution, and both were independent of the amount of solid phase present *(5)*.

Berridge *(7)* suggested that the method of crystallization described by Berridge and Woodward *(10)* might not work with all rennet extracts. He *(9)* attributed the failure of such rennin preparations to crystallize to the presence of contaminating impurities which defied removal, but which could be detected electrophoretically.

Hostettler and Stein *(18)* concluded that the nature of the starting material as well as the method of purification had a marked influence on the properties of crystalline rennin.

Needle-shaped crystalline rennin was prepared by Hankinson *(16)* and DeBaun *et al. (14)* from rennet extract available in the United States. However, electrophoretic patterns published by DeBaun *et al. (14)* indicated considerable heterogeneity in phosphate buffers at ionic strengths of 0.1 and 0.2. Oeda and Kasai *(24)* also produced needle-shaped crystalline rennin, for which they reported electrophoretic homogeneity in phosphate buffer at an ionic strength of 0.14.

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It was the purpose of this work to prepare crystalline rennin from rennet extract available in the United States, and to study it under conditions of electrophoresis likely to give greater resolution than had been previously reported.

EXPERIMENTAL METHODS

Rennin activity. Rennin activity was meaured by comparing the milk-clotting ability of unknown rennin solutions with that of a standard-strength rennet extract received monthly from a commercial rennet manufacturer.[3] Each fresh standard was in turn checked against the standard of the previous month, using a substrate in which 60 g. low-heat nonfat dry milk (NDM) was dissolved in 500 ml. of 0.01 *M* calcium chloride *(6)*. The same lot of NDM was used to prepare all substrates which, after reconstitution, were allowed to equilibrate for 20 hr. at 2° C. before use. Testing was carried out at 30° C. on 25-ml. portions of substrate in 125-ml. wide-mouthed bottles. One ml. of diluted enzyzme was added, and the clotting time measured in revolutions (16 r.p.m.) on the apparatus described by Sommer and Matsen *(26)*. The first appearance of visible flecks on the moving glass surface was taken as the end-point. The standard rennet extract, arbitrarily given a value of 100 R.U./ml, had an activity such that one milliliter of a 1:250 dilution produced visible clotting of the substrate in 100 ± 5 revolutions. Variation in the response of different lots of substrate to rennin accounted for some variation in clotting time. Consequently, whenever rennin solutions were tested, the activity of the standard was measured simultaneously on the same substrate.

Dilutions of unknown rennin solutions were made with distilled water, and adjusted so they gave coagulation times within ten revolutions of a 1:250 dilution of the standard.

Rennin activities were calculated as:

$$\text{R.U/ml} = 100 \frac{T_s}{T_u} \times \frac{C_s}{C_u}$$

Where

R.U.	= rennin units
T_s	= coagulation time of standard
T_u	= coagulation time of unknown
C_u	= concentration of unknown
C_s	= concentration of standard

Nitrogen determinations. Nitrogen content of rennin fractions was measured by a micro-Kjeldahl procedure *(17)*.

Purification and crystallization of rennin. Rennin was concentrated and purified from Chr. Hansen's rennet extract by a modification of the procedure of DeBaun *et al. (14)*. The original extract contained no added color or flavoring, and only 0.1% sodium benzoate as a preservative. The strength of this rennet had not been standardized, and most of the lots received had greater activity than commercial cheese rennet.

[3] Rennet standard supplied by Chr. Hansen's Laboratory, Inc., Milwaukee, Wis.

The purification and crystallization procedure is outlined below:

1. Rennet extract at room temperature was adjusted to pH 5.0 with 3 *N* HCl, and saturated with sodium chloride. A precipitate formed (Precipitate I) which was separated from the supernatant by centrifuging for 45 min. at 5,000 × gravity (max.) in an International refrigerated centrifuge with a No. 845 angle head.
2. Precipitate I was dissolved in distilled water, and diluted to one-half the original volume. The solution was adjusted to pH 6.3 with 0.1 *N* NaOH and filtered.
3. Steps 1 and 2 were repeated until four salt precipitations had been made (Precipitates I, II, III, and IV). The dissolved Precipitate IV was diluted to the same volume as the solution of Precipitate III.
4. Potassium alum (1.0 g. per 1000 ml. rennin solution), dissolved in a small amount of water, was added to the solution of Precipitate IV, and immediately neutralized to pH 6.3 with 0.1 *N* NaOH. An aluminum hydroxide gel formed which was removed by centrifuging for 15 min. at 5,000 × gravity (max.). The alum supernatant was retained.
5. Rennin in the alum supernatant was precipitated by adjusting to pH 4.6 with 0.1 *N* HCl and saturating with sodium chloride, and separated by centrifuging in a refrigerated centrifuge for 45 min. at 5,000 × gravity (max.), (Precipitate V.). The supernatant was discarded.
6. Precipitate V was dissolved in a minimum of 0.05 *M* sodium phosphate buffer at pH 6.8 and dialyzed at 2° C. against Berridge's *(5)* salting-in buffer (50 g. $MgCl_2 \cdot 6\ H_2O$ plus 15 g. $CH_3COONa \cdot 3\ H_2O$ per liter, adjusted to pH 5.4 with 10 *N* H_2SO_4).
7. Crystals formed after a few days and continued to deposit for about 4 wk. Crystal formation was materially hastened by adding a few seed crystals from a previous batch.
8. The crystals were washed twice in the crystallizing buffer and twice at pH 5.6 in 0.05 *M* acetate buffer containing 0.3% sodium chloride.

RESULTS

Analyses of fractions during the purification and crystallization of a representative batch of rennin are given (Table 1).

The data in Table 1 show a 20-fold increase in specific activity of the crystals over the original rennet. Other preparations showed increases of 20- to 23-fold, depending on the purity of the original extract. Attempts to further increase the specific activity by recrystallization have not been successful.

Figure 1 shows a photomicrograph of crystalline rennin. The crystals are similar in appearance to those described by Berridge *(4, 5)*, and actually have considerable depth. Many of them are as deep as they are wide, resembling blocks more than plates.

TABLE 1

Analyses of fractions during purification and crystallization of rennin

Fraction	Volume	Activity	Total activity	Activity recovered	Nitrogen	Specific activity
	(ml.)	*(R.U./ml)*	*(R.U.)*	*(%)*	*(mg/ml)*	*(R.U./ mg N.)*
Original extract	3,870	100	387,000	100	5.01	20
Solution of						
Precipitate I	1,960	150	294,000	76	0.96	156
Precipitate II	955	263	251,200	65	1.07	246
Precipitate III	550	394	216,700	56	1.48	266
Precipitate IV	477	459	218,900	57	1.56	294
Alum supernatant	499	265	132,200	34	0.78	340
Solution of						
Precipitate V	72	1,718	123,700	32	4.81	357
Crystals[a]			62,270	16		401

[a] A portion of the crystals was dissolved in 0.2 *N* acetic acid, dialyzed against 0.05 *M* phosphate buffer at pH 6.8, and recrystallized by dialyzing at 2° C. against Berridge's *(5)* buffer. The specific activity of the recrystallized rennin was 399 R.U/mg *N*.

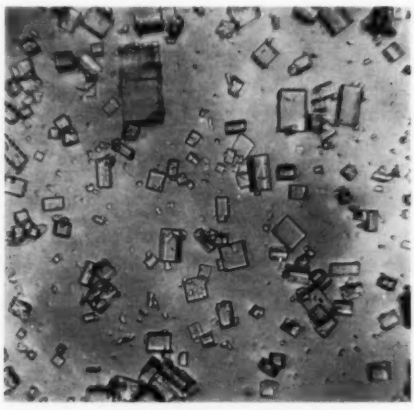

FIG. 1. Crystalline rennin × 180.

Electrophoresis of crystalline rennin. Electrophoresis was carried out in 0.1 and 0.017 *M* sodium phosphate buffer at pH 6.8. The ionic strengths were 0.2 and 0.033, with specific conductivities at 0° C. of 4.8×10^{-3} and 1×10^{-3} $ohms^{-1}\text{-}cm.^{-1}$, respectively. At 0.2 ionic strength the buffer approximated that employed by Alais *(1)* in his electrophoretic analysis of crystalline rennin. An ionic strength of 0.033 was then used to achieve greater resolution by providing a higher ratio of protein concentration to ionic strength, and by enabling the use of a higher field strength in the electrophoresis cell.

Figure 2 shows electrophoretic patterns of rennin at a protein concentration of 0.6% in sodium phosphate buffer at an ionic strength of 0.2. The ascending pattern shows evidence of a small amount of material apart from the leading boundary. Using an assumed refractive increment of $dn/dc = 1.9 \times 10^{-3}$ (c = gram per 100 ml.), and counting the Rayleigh fringes intercepted by the

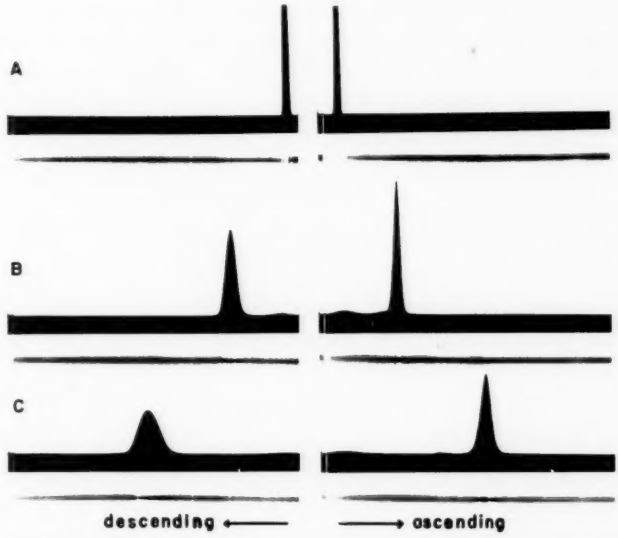

FIG. 2. Electrophoretic patterns of crystalline rennin dissolved to a protein concentration of 0.6% in sodium phosphate buffer at pH 6.8 and 0.2 ionic strength. The field strength was 4.34 volts/cm, with a current of 16 ma. (A) initial boundary, (B) 118 min., (C) 311 min.

horizontal, the amount of material represented by the trailing boundary was calculated to be about 3.8%.

Figure 3 illustrates an electrophoretic pattern of the same material at a protein concentration of 0.7% in pH 6.8 sodium phosphate buffer at an ionic strength of 0.033. This figure shows the presenec of at least four, and possibly five, electrophoretically distinguishable components in the ascending pattern.

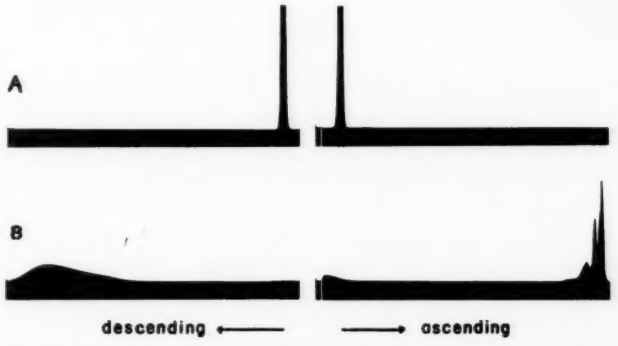

FIG. 3. Electrophoretic patterns of crystalline rennin dissolved to a protein concentration of 0.7% in sodium phosphate buffer at pH 6.8 and 0.033 ionic strength. The field strength was 10.46 volts/cm, with a current of 8 ma. (A) initial boundary, (B) 127 min.

These results have led to the conclusion that rennin preparations can not be considered homogeneous on the basis of their crystalline form or their electrophoretic behavior in phosphate buffers of high ionic strength.

Crystalline rennin supplied by Foltmann *(15)* gave similar results when run under the same conditions of electrophoresis.

DISCUSSION

The effect of reducing the ionic strength on improving the electrophoretic resolution of rennin is similar to that reported by Cann *(12)*, in his studies on the resolution of ovalbumin in electrophoresis. This effect may be attributed to the higher field strength which can be used with a buffer of low conductivity, and to an increase in the ratio of protein concentration to ionic strength. In the absence of diffusion, resolution of components in electrophoresis is proportional to the product of field strength and time. Since boundary spreading by diffusion is proportional to the square root of time, it follows that resolution in ideal electrophoresis is proportional to field strength times the square root of time *(3)*. Consequently, increased field strength gives greater resolution, since more rapid movement of the boundaries allows less time for diffusion.

Alberty *(2)* points out that in the electrophoresis of proteins descending peaks are usually, but not always *(20)*, broader and shorter than the corresponding ascending peaks. This is due to deviation from ideality, which becomes more pronounced as the ratio of protein concentration to ionic strength is increased. Longsworth *(20)* showed that these boundary characteristics resulted from differences in conductivity and pH between the leading and trailing edges of the boundaries. When the conductivity effect predominates, the field strength along the leading edge of the ascending boundary is less than along the trailing edge. Therefore, the molecules along the trailing edge move more rapidly than those on the leading edge and the boundary is sharpened. A broadening of the peak occurs on the descending side, where the field strength is greater along the leading than on the trailing edge of the boundary. The pH effect usually opposes the conductivity effect above the iso-electric point of the protein, but in most instances it does not predominate *(2)*.

The results of this work point to the interesting possibility that either: *(a)* more than one electrophoretically distinguishable component in crystalline rennin possesses milk-clotting activity, or *(b)* further fractionation should achieve a considerably greater increase in specific activity. Since at least four electrophoretic components were observed, both *(a)* and *(b)* may be correct.

There are several known instances of more than one electrophoretic form of an active enzyme. Examples are enolase *(21)* and chymotrypsin *(22)*. Of these, the case of chymotrypsin is particularly relevant. Jacobsen *(19)* found that π- and δ-chymotrypsin appeared to be different stages in the activation of a single pro-enzyme, chymotrypsinogen. It was later shown that these two active enzymes possessed different electrophoretic mobilities, and that crystalline α-chymotrypsin *(23)* represented an electrophoretically heterogeneous mixture *(22)*.

ACKNOWLEDGMENT

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INFLUENCE OF HYDROGEN AND CALCIUM ION CONCENTRATIONS, TEMPERATURE, AND OTHER FACTORS ON THE RATE OF AGGREGATION OF CASEIN

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SUMMARY

The rates of aggregation of 1% whole casein, α-casein, mixtures of α- and β-casein, and pepsin-paracasein, produced by the addition of calcium chloride at pH 6.3, have been studied at 25, 30, and 35°; the rates of aggregation of β-casein alone have been studied at 30 and 31°. Temperature coefficients for the rate of aggregation are quite high (> 14 per 10°). Similar studies were performed with casein to which phosphate was added, with casein plus citrate, with a mixture of α- and β-casein (3:1), and with casein aggregated without calcium chloride at pH 5.5. In addition, the aggregation of whole casein by $CaCl_2$ has been studied from pH 7.9 to 5.5, at concentrations of casein of 0.2 to 1.5%, and the effect of several concentrations of sodium chloride on the aggregation determined.

A study *(17)* of the effect of temperature on the precipitation of casein with calcium chloride has shown that the development of turbidity is time-dependent and largely reversible. In the present report, the initial stages of casein aggregation, implemented by calcium chloride or by hydrogen ions (pH 5.5), have been studied turbidimetrically at 25 to 35°. Effect of concentrations of the components, effect of buffer type and concentration, effect of sodium ion, phosphate ion, and citrate ion also have been studied. The caseins studied have been whole casein, α-casein, β-casein, and pepsin-paracasein.

MATERIALS AND METHODS

Whole casein. The casein was precipitated from skimmilk by acidification of the milk to pH 4.5 with *N* HCl. The precipitate was washed four times with water, and redissolved. The precipitation and washing were repeated twice *(6)*. The precipitated casein was dried with absolute ethanol and ether.

α-Casein and β-casein. These caseins were prepared from whole casein by fractionation in aqueous urea solutions *(7)*.

Pepsin-paracasein. Paracasein was prepared from whole casein with pepsin. A 100-ml. portion of 2% sodium caseinate solution, pH 6.5, containing 15.0 m*M* $CaCl_2$ per liter, was treated with 2 mg. crystalline pepsin at 30°. A clot formed at the end of 5 min. The suspension was heated for 15 min. at 100° to inactivate the pepsin. The casein clot was washed at pH 4.7 to reduce the calcium concentration and finally dissolved at about pH 7 and reprecipitated several times at pH 4.7. The paracasein was finally dried with absolute ethanol and ether.

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[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

All of the caseins were stored in a desiccator at a relative humidity that maintained the moisture content at 10%. The concentrations given are for moisture-free products.

Preparation of solutions. In most instances, the casein solutions were freshly prepared for each experiment. However, it has been found that a stock 4% solution of sodium caseinate, pH 6.5, remained stable for at least five days, kept at 7°. In preliminary aggregation experiments with unbuffered solutions, the rate curve was difficult to reproduce. Satisfactory reproducibility was obtained when the solutions were buffered. Solutions were freshly prepared by dissolving the casein in buffer, or the stock solution of casein was diluted with an appropriate amount of buffer, as described for each experiment. Because of the strong solubilizing effect of sodium ions in comparative systems, for example where NaOH was used to adjust the pH, it was necessary to keep the sodium ion concentration constant, which was done by adding sodium chloride.

Turbidity measurement. The extinction of light caused by the turbidity was read in a Beckman Model B spectrophotometer[2] at a wave length of 600 mμ in glass tubes 18 mm. O.D., 16 mm. I.D., selected for optical uniformity. The temperature of the cell was maintained by circulating water maintained at the specified temperature $\pm$ 0.2. Measurements in this instrument with a suspension of polystyrene latex, diameter 0.13 μ, which superficially resembles the casein aggregates, were linear with concentration for extinction values up to 1.3.

RESULTS

The rates of aggregation of whole casein at 30.0°, pH 6.30, in cacodylate buffer, ionic strength (μ) of 0.030, with several concentrations of $CaCl_2$, are shown in Figure 1 (solid lines) as extinction (E) values. Also shown in this figure is the strong effect of NaCl in reducing aggregation (dash lines). The rates of aggregation are not described by any simple rate law, but with 4.5 to 7.0 m*M* $CaCl_2$ per liter the curves are parabolic and, when E^2 is plotted aginst time, straight lines are obtained. At lower concentrations of calcium chloride, there is an initial lag period in the aggregation similar to that shown at an ionic strength of 0.065. The rates of aggregation were also determined for a range of casein concentrations with several calcium chloride concentrations. The results are given in Figure 2 as derived rates; namely, the change in E^2 with time. The effect of temperature on the aggregation of whole casein is shown in Figure 3. This figure shows also the effect of temperature on the rate of aggregation of a 3:1 mixture of α- and β-caseins.

The rates of aggregation obtained at different pH values are shown in Figure 4. Overlap of the pH values with the three buffers used showed that there were no marked specific buffer effects. In view of the effect of pH on calcium aggregation, the aggregation in the isoelectric region without calcium ion was measured. The results are shown (in Figure 5), together with data showing

[2] Mention of commercial products does not imply recommendation by the U. S. Department of Agriculture over similar products not mentioned.

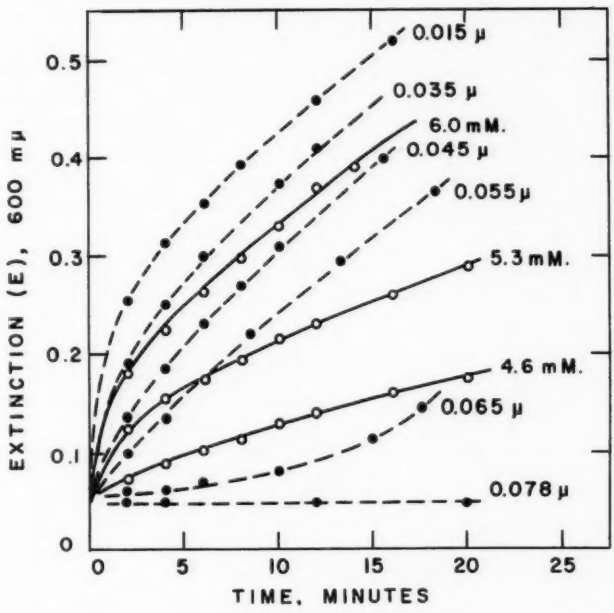

FIG. 1. The rate of aggregation of sodium caseinate (1.0%) in cacodylic acid (0.05 *M*)-NaOH (0.030 *M*) buffer, pH 6.28, by the addition of calcium chloride as measured by the extinction at 600 mμ at 29.8° (solid lines). The numbers indicate the concentrations of calcium chloride in m*M* per liter. Also shown (dashed lines) is the effect of soduim chloride concentration on the aggregation, all at 6.0 m*M* $CaCl_2$ per liter, with the buffer one-half as concentrated (0.015 *M*). Numbers indicate ionic strength. The lowest (0.015) contains buffer only, the others contain NaCl in addition.

the effect of temperature on this aggregation. This aggregation is inhibited by NaCl (Figure 5), just as is the $CaCl_2$ aggregation (Figure 1).

Phosphate is present in the natural calcium-casein complex in milk. Further, it has been found to prevent or greatly retard *(17, 18)* the reversal at low temperatures of the aggregation of calcium caseinate brought about at high temperatures. Accordingly, the influence of phosphate on the rate of aggregation was of interest. The results are shown (in Figure 5) for temperatures of 25, 30, and 35°. The phosphate not only reduces the amount of aggregation obtained in a given time, as one would expect, by the binding of calcium ions *(5)* (at pH 6.3, less than 30% of the phosphate is present as divalent phosphate, the ion which binds the calcium), but it also changes the shape of the rate curve, and greatly reduces the effect of temperature on the aggregation. These changes are probably due to the formation of the calcium phosphate–calcium caseinate complex. The effect of sodium citrate on the aggregation was also studied for comparison, for the result presumably would be due solely to the reduction in the calcium concentration by the binding to the citrate. The results are shown (in Figure 6)

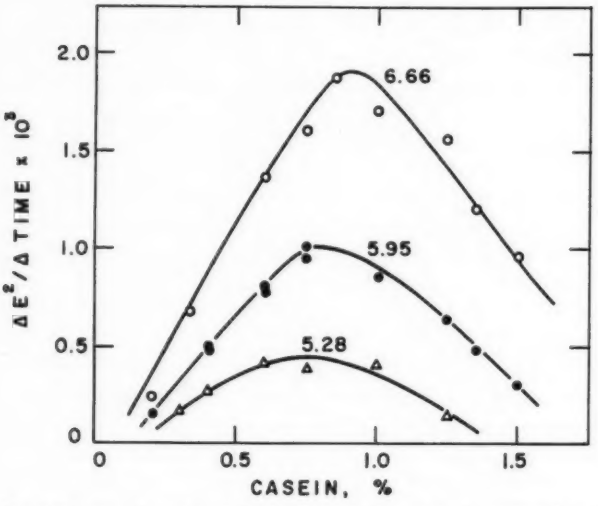

FIG. 2. The influence of casein concentration on the rate of aggregation ($\Delta E^2/\Delta$ time) of casein by calcium chloride. The buffer was the same as for Figure 1. The numbers indicate the concentration of calcium chloride in m*M* per liter.

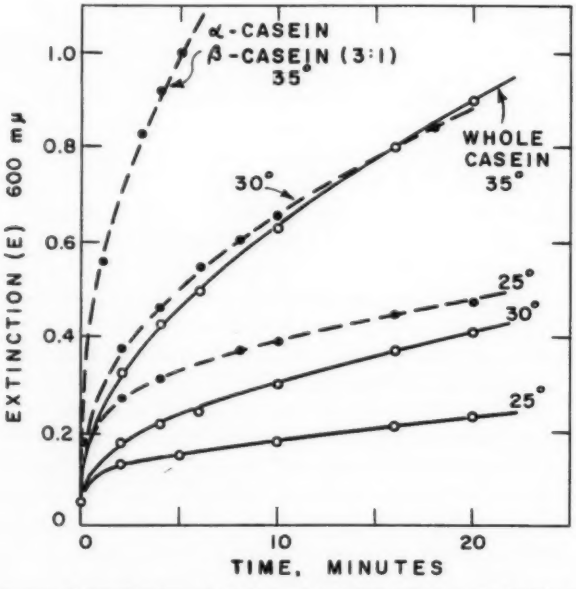

FIG. 3. The rate of aggregation of whole casein (solid lines) in cacodylate buffer, pH 6.33 at 25, 30, and 35° with 6 m*M* $CaCl_2$ per liter. Also shown is the rate of aggregation of a 3:1 mixture of α- and β-casein (dashed lines) under the same conditions.

for 25, 30, and 35° and it is apparent that the effects are what one would expect from a reduction in the calcium concentration.

The aggregation of an α-casein preparation at 25, 30, and 35° in the presence of calcium chloride is shown (Figure 7). In the period of time studied, the aggregation is linear. Two concentrations of calcium chloride were used at 30°. A comparison of the larger of these, 4.5 mM per liter, with the 4.6 mM concentration in Figure 1, shows that the aggregation of α-casein is more sensitive to calcium concentration than is whole casein. A quantitative expression of the sensitivity has been chosen as the concentration of calcium chloride required to give an extinction of 0.5 in 30 min. at 30°. On this basis, the sensitivity of whole casein is 6.0. The α-casein preparation used in the present study had a sensitivity number of 4.1. Other preparations, differing as much as tenfold in the concentration of calcium chloride required to precipitate them,[3] had sensitivity numbers much the same; namely, from 3.9 to 4.7. The differences in precipitation of α-casein are attributed to differences in content of K-casein *(15)*.

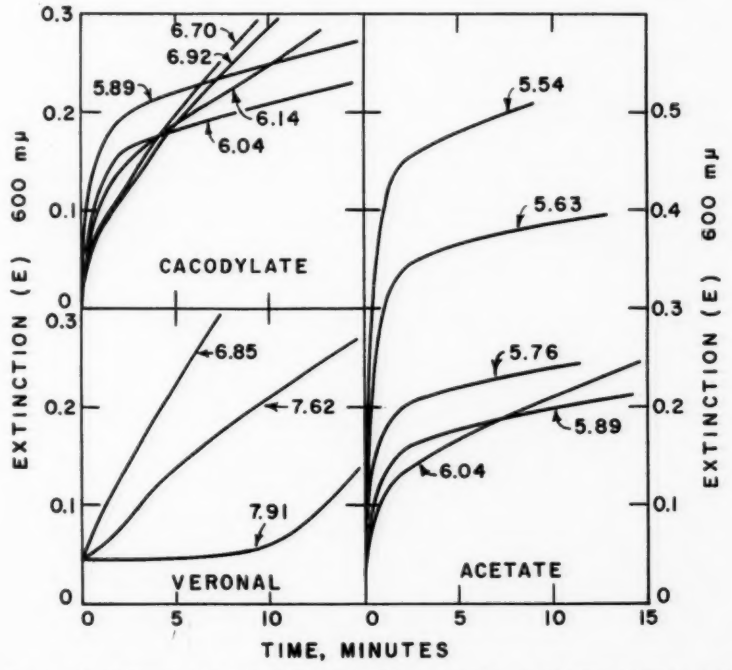

FIG. 4. The influence of pH on the aggregation of casein by calcium chloride (5.95 mM per liter) in veronal (0.05 M sodium salt plus HCl), cacodylate (0.05 M acid plus NaOH), and acetate (0.05 M sodium salt plus HCl) buffers at 29.7°. The total sodium ion concentration in each was 0.050 M; sodium chloride was added when necessary to attain this concentration. The numbers on the graph give the pH values.

[3] Unpublished studies.

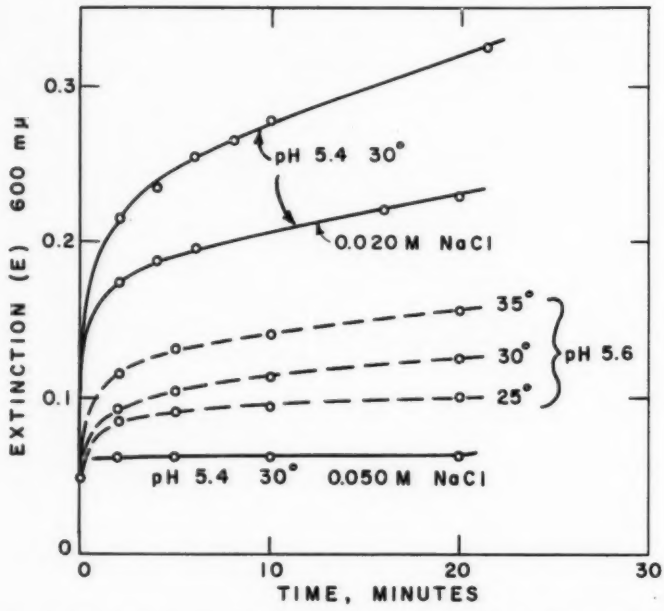

FIG. 5. The rate of aggregation of casein in the isoelectric region at pH 5.4 and 5.6 without calcium chloride, at 25, 30, and 35°. Also shown is the effect of NaCl on the aggregation. The low pH was obtained by adding 2.0 ml. of 0.1 *M* cacodylate buffer, pH 5.06 (obtained by acidifying 20.0 ml. of the 0.1 *M*, pH 6.53 cacodylate buffer with 1.10 ml. of 1.0 *N* HCl) to 4.0 ml. of the casein-containing solution in the spectrophotometer. The final pH of 5.6 was obtained when 0.33 ml. of 0.1 *N* NaOH was used to dissolve the casein (pH about 6.5); for the final pH of 5.4 only 0.23 ml. of NaOH was used.

The results of both precipitation and rate of aggregation experiments suggest that K-casein influences not the amount of aggregate formed but its stability.

β-Casein was difficult to study because of its extreme sensitivity to temperature and its dependence on calcium chloride concentration. Some of the results are shown (Figure 8). At 30° with 8 m*M* $CaCl_2$ per liter, the solution remained clear; with 10 m*M* the aggregation shown was obtained, and with 11 m*M* a dense white suspension with a gummy precipitate was obtained. Only a 1° temperature span could be studied quantitatively in the spectrophotometer. Any greater increase in temperature gave a white suspension and gummy precipitate. One curve is shown for whole casein (dash line), which at this particular temperature (30°) is more sensitive to $CaCl_2$ than is β-casein.

The aggregation of pepsin-paracasein by calcium chloride at 25, 30, and 35° is shown (Figure 9). Paracasein is more sensitive to calcium ions than is casein and in these experiments the concentration of $CaCl_2$ was 4.0 m*M* per liter, a concentration that gives a barely detectable aggregation of casein under the same conditions.

DISCUSSION

The primary emphasis in the present study was to determine the rate at which casein aggregates in the presence of calcium ions or in the isoelectric region (pH 5.5). The size of the aggregates was of less interest; hence, a spectrophotometric method that would give comparative values was considered satisfactory. The aggregates studied are quite large and give an opalescent, milky appearance to the casein solution. Under the conditions studied, the aggregates are stable colloids, however, and no precipitation or sedimentation occurred during the period of study. Consideration of the results shows that in a general way the aggregations were of two types: (*1*) aggregation was almost instantaneous when $CaCl_2$ was added; (2) aggregation was gradual when $CaCl_2$ was added. β-Casein, pepsin-paracasein, casein plus phosphate, and casein plus acid (no $CaCl_2$) were of the first type, whereas whole casein, α-casein, and casein plus citrate were of the gradual type.

The aggregation rate curves obtained with whole casein and $CaCl_2$ are very similar to curves obtained by Dyachenko and Vlodavets *(4)*. These authors prepared their casein *(3)* by solution at pH 3.0 and reprecipitation with NaOH.

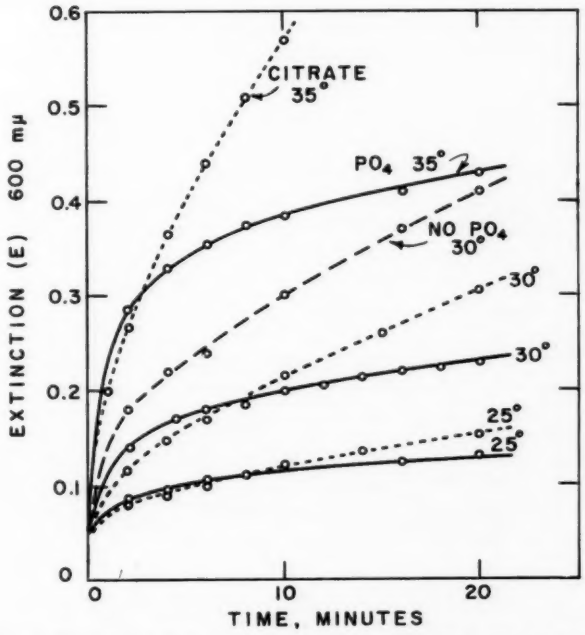

FIG. 6. Rate of aggregation of whole casein in cacodylate buffer, pH 6.33 at 25, 30, and 35° with 6 m*M* $CaCl_2$ per liter, in the presence of 5 m*M* of phosphate per liter. Also shown is the rate of aggregation in the same systems with 0.5 m*M* sodium citrate per liter replacing the phosphate. The aggregation of casein without phosphate or citrate at 30° is shown for comparison.

It was surmised that the procedure used would remove the β-casein from the α-casein *(14)*, but unless the temperature was low (about 2°) and the casein solution dilute (0.2 to 0.3%), no separation of α- and β-caseins would occur. The shape of their aggregation curves suggests that they have whole casein and not α-casein.

Dyachenko and Vlodavets report *(4)* that there is an optimum $CaCl_2$ concentration for maximum aggregation and that concentrations of $CaCl_2$ greater than the optimum effect a reduction in the size of the aggregates. In unbuffered systems at pH 6.0, containing 0.018 mg. casein per milliliter at 40°, the optimum $CaCl_2$ concentration was 0.01 *M*. The whole casein used in the present studies with a cacodylate-buffered system, pH 6.3, containing 0.5 mg. casein per milliliter at 30°, showed no solubilization effect with concentrations of $CaCl_2$ of 0.1 to 1.0 *M*. This range of calcium concentrations covers the calcium : casein ratios showing the reported solubilization *(4)*.

The principal function of the calcium in the casein aggregation is probably through binding to the casein to reduce the net charge, so that it is comparable to isoelectric casein. Calcium, however, may also have a specific contribution to the aggregation, perhaps through hydration, for at alkaline pH values, potassium salts of casein increase in solubility with increase in temperature, whereas the calcium salts decrease in solubility *(19, 20)*.

The effect of various ions on the aggregation of casein is complex. Sodium

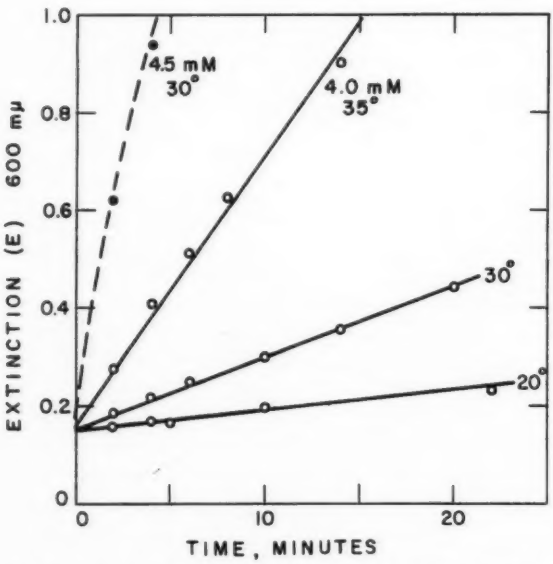

FIG. 7. Rate of aggregation of α-casein (1%) in cacodylate buffer at pH 6.33 at 25, 30, and 35°. At each temperature, the concentration of calcium chloride was 4.0 m*M* per liter. One curve is shown for 4.5 m*M* $CaCl_2$ per liter at 30°.

chloride decreases the activity of the calcium ion, and sodium ion probably is competitive with calcium ion *(18)*. The effect of sodium chloride on the calcium-free, isoelectric aggregation shows also that salts must exert an electrostatic interaction with the charged protein ion, that is, a salting-in effect. This occurs also with the calcium aggregates. Cacodylate ion reduces the rate of aggregation somewhat, compared with chloride ion. This is apparent in Figure 1, for the experiments with 6 m*M* $CaCl_2$ per liter and 0.030 μ (the variable $CaCl_2$ experiments are all for μ of 0.030). With buffer, the E value is 0.33 for 10 min.; when one-half the buffer is replaced with NaCl, the E value is 0.37 at 10 min. A col-

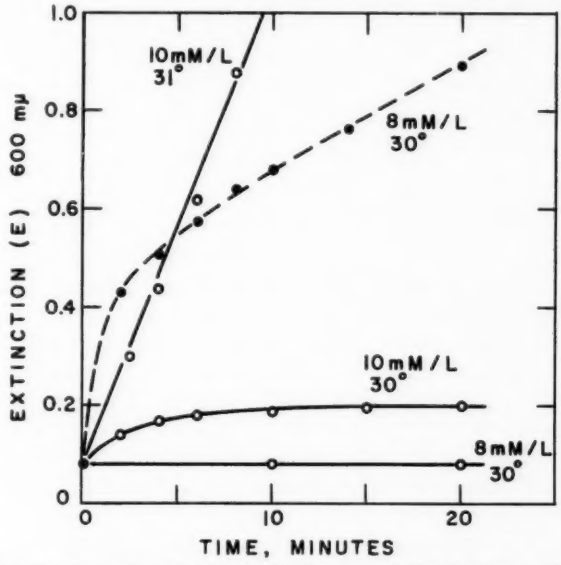

FIG. 8. Rate of aggregation of β-casein (1%) in cacodylate buffer at pH 6.33 at 30 and 31° with 10 m*M* $CaCl_2$ per liter. No aggregation is obtained with 8 m*M* per liter. The aggregation of whole casein (dashed line) with this concentration of $CaCl_2$ is shown for comparison.

lidine buffer (0.025 *M*, pH 7.0), on the other hand, exerted an enhancing effect on the aggregation, presumably due to the positively charged collidinium ion. The collidinium ion could not substitute for calcium ion, however, for even with a collidine concentration of 0.05 *M*, no aggregation occurred without $CaCl_2$. The effect shown by the collidinium ion may be exerted by other ammonia type ions, since it has been observed *(9)* that ammonium salts are less inhibitory to casein coagulation by pepsin than are sodium and potassium salts. The negatively charged veronal ion is slightly inhibitory to the aggregation, an effect which might be due to the binding of calcium ions.

The calcium : casein ratio is a factor which influences the rate of aggregation (Figure 2). This follows from the laws of mass action, which apply to the bind-

ing of calcium ions by casein *(2)*. In solutions containing not more than 1% casein and 6.66 m*M* $CaCl_2$ per liter, the neutralization of charge on the casein molecule brought about by the binding of Ca^{++} is maximal, and under these circumstances, rate of aggregation is determined by the number of casein molecules per unit volume, *i.e.*, the concentration. With lower concentrations of calcium, the concentration of casein giving a maximum rate of aggregation is somewhat lower. Beyond this maximum, the rates decrease, presumably because the amount of calcium available to reduce the charge on each casein molecule becomes increasingly inadequate.

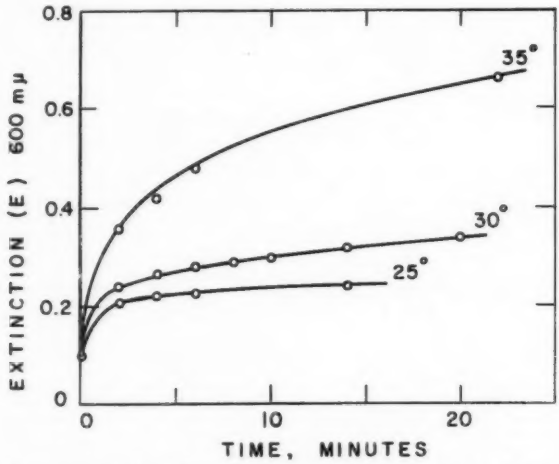

FIG. 9. Rate of aggregation of pepsin-paracasein in cacodylate buffer, pH 6.33 at 25, 30, and 35°, with 4.0 m*M* $CaCl_2$ per liter.

The effect of pH on the calcium aggregation of casein is quite complex. In the system studied (1% casein, 5.95 m*M* $CaCl_2$ per liter) at pH 7.9, there is an initial lag period in the aggregation. This is also observed at pH 6.3 when calcium concentration is low and in systems to which considerable NaCl is added (Figure 1). As the pH is lowered, the rate of aggregation increases rapidly and, for a limited range, the curves are parabolic. Below pH 6, however, the curves are rectilinear. Due to this change, it is difficult to compare the curves; however, a comparison of the times required to obtain an extinction of 0.2 shows that there is a minimum at pH 6.7, followed by a maximum at pH 5.9, and a continuous decrease at lower pH values. The nature of the pH influence is not understood, but it may reflect changes in hydration with the aggregate being least hydrated at low pH values.

The temperature coefficient of the aggregation of casein by $CaCl_2$ is of considerable interest. It is obvious from the behavior of β-casein that the temperature coefficient of aggregation is extremely high. The linear aggregation curves obtained with α-casein provide data for calculation of the temperature coefficient.

The temperature coefficient for the aggregation reaction can be calculated from the integrated form of the Arrhenius equation *(12, 13)*:

$$R_2/K_1 = e^{\frac{\Delta H(T_2-T_1)}{R(T_2T_1)}}$$

K_2 *and* K_1 are the rate constants at two temperatures, ΔH a constant equivalent to the heat of activation of the reaction, R the gas constant of 1.99 calories per mole, and T_2 and T_1 the absolute temperatures. When the temperature range is relatively short, the product T_2T_1 can be regarded as a constant and the temperature coefficient takes the form $K_2/K_1 = e^{k(T_2-T_1)}$. This form was used by Berridge in studies on the effect of temperature on the clotting of paracasein *(1)*. A plot of log K (rate) for α-casein versus temperature gave a straight line, the slope of which gave the k value. The value of e^k gives the temperature coefficient for a 1° interval; e^{10k} gives the value for a 10° interval. The 10° temperature coefficients obtained from e^{10k} are identical with the values of the ratios $K_{35°}/K_{25°}$. The 1° temperature coefficient for α-casein is 1.30; the 10° temperature coefficient is 14. Similar calculations with the whole casein data with the linear rates obtained from E^2 versus time plots gave 1 and 10° temperature coefficients of 1.35 and 19, respectively. Thus, these aggregations are characterized by high temperature coefficients.

Berridge *(1)* studied a related phenomenon, the clotting time of para-casein with calcium ions, and compared the inverse of the time required for clotting at several temperatures. The 1° temperature coefficients at 26 to 38° range from 1.3 to 1.6, depending on the sample of skimmilk used. The shape of the present aggregation curves of pepsin-paracasein does not permit a similar end-point comparison, but the 1° temperature coefficients of calcium ion aggregation of casein, in general, is of the same magnitude as Berridge's values. Pyne *(10)* has reported the 1° temperature coefficients of clotting of calcium caseinate to be 2.0/1°, without giving any experimental details. He also observed, analogously with the present observations, that the addition of phosphate reduces the temperature coefficient about 20%, to a value with phosphate of 1.6/1°. Berridge concluded from the high-temperature coefficients and a heat of activation of the magnitude associated with denaturation that the clotting was a denaturation phenomenon. There are many aspects of the clotting reaction, however, that make the denaturation hypothesis appear to be untenable. The aggregation reactions shown by the caseins other than paracasein are probably comparable reactions. In the case of β-casein, the heat of activation of the reaction is extremely high. Further, the aggregation is partly reversed by lowering the temperature, or it can be reversed by removing the calcium. If the phenomenon was a denaturation involving solely hydrogen cross-binding, as its reversibility suggests, one might expect an increase in temperature to decrease the aggregation, as is observed for gelatin *(8)*. Casein in acid solution (pH 1.5) shows *(3)* an aggregation of this type; that is, the aggregation decreases as the temperature is raised (20 to 84°). The magnitude of this temperature effect was considered to be consistent with hydrogen bond aggregation. Thus, the clotting of casein probably resides in some phenomenon other than denaturation. The evidence suggests that in

clotting the usual aggregation of casein by calcium has been enhanced by the removal of a stabilizing protein by the action of rennin to give the less stable paracasein *(16)*.

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DIRECT DETERMINATION OF CITRIC ACID IN MILK WITH AN IMPROVED PYRIDINE-ACETIC ANHYDRIDE METHOD

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SUMMARY

The determination of citric acid with pyridine and acetic anhydride has been investigated at reaction temperatures from 17 to 60° C. The optimum proportions of pyridine, acetic anhydride, water, and acetic acid for maximum color intensity and stability are given for each temperature. The procedure has been modified to eliminate the violent nature of the reaction, even when the analysis is done at a reaction temperature of 60° C. Details of a method for the determination of 25–200 μg. of citric acid, at a reaction temperature of 32° C., are presented. In comparison with previously published methods based on the reaction, the recommended technique results in improved sensitivity, stability, and reproducibility without requiring careful timing.

The method has been successfully applied to the routine analysis of milk and milk products. Milk and serum can be analyzed directly, after suitable dilution. Corrections for the interference caused by fat in homogenized milk, and by trichloroacetic acid in T.C.A. serum, can be made easily.

Results of direct analysis of milk were from 5 to 15% higher than those for the corresponding sera and are believed to represent the true values for the citric acid content of milk.

Several methods for the estimation of citric acid depend on the Fürth and Herrmann reaction *(4)*, in which color is formed in the presence of pyridine and acetic anhydride. Saffran and Denstedt *(10)* applied this reaction to the colorimetric determination of citric acid in animal fluids and tissues, whereas Babad and Shtrikman *(1)* modified this procedure to estimate citric acid in milk serum. In both procedures, the sample was held for 10 min. at 60° C. in the presence of an excess of acetic anhydride, pyridine was added, and the mixture held a further 40 min. at 60° C., then cooled in an ice-bath. The two heating periods were accurately timed to minimize variability. Reducing the amount of water added with the sample was found to lower the intensity of the yellow color, but enhanced reproducibility *(1)*. Temperatures higher than 60° C. decreased color intensity and stability, while lower temperatures gave greater sensitivity but required longer holding periods for full color development *(10)*. Addition of pyridine to the hot solution caused a vigorous reaction that made it difficult to keep the tubes stoppered during the subsequent holding period *(1)*. Reinart and Nesbitt *(8)* modified the method to reduce the violence of the reaction at 60° C., whereas Murthy and Whitney *(6)* recommended the use of glass stoppers instead of rubber stoppers, to prevent possible contamination of the sample. However, even by rigid adherence to all of these recommendations, reproducible results were difficult to obtain.

The present investigation was undertaken to study factors affecting color development, with emphasis on lower reaction temperatures, and led to a procedure at a reaction temperature of 32° C. for the direct determination of citric acid in milk.

[1] Manuscript received June 23, 1958. Contribution from the Division of Applied Biology, National Research Council, Ottawa, Canada.

DEVELOPMENT OF THE METHOD

A preliminary study of the basic aspects of the reaction was made. It was found that the reaction was strongly exothermic, that a constant reaction temperature was essential to reproducibility, and that rapid color formation at temperatures below 60° C. could be induced by introducing more water with the sample. However, water and acetic anhydride concentrations were difficult to control if they were allowed to interact before the addition of pyridine. On the basis of these observations, pyridine was added to the sample prior to acetic anhydride and, as color development was not affected by the time interval between the two additions, the need for timing the pyridine step was obviated. All reagents were added at room temperature and the tubes placed in a water bath immediately after adding the anhydride. In this manner, the vigorous nature of the reaction was completely eliminated even at a reaction temperature of 60° C., and it was no longer necessary to stopper the tubes. This modified procedure was, therefore, adopted in the following studies.

The effect of concentration of pyridine and of water on changes in color intensity at 420 mμ with time was studied at fixed concentration of the other reagents. Glacial acetic acid was added as necessary to maintain constant volume and acted only as a diluent. The results obtained at a reaction temperature of 32° C. (Figure 1, A and B) show that increasing concentrations of water or pyridine to an optimum value increased the rate of color formation, but that an

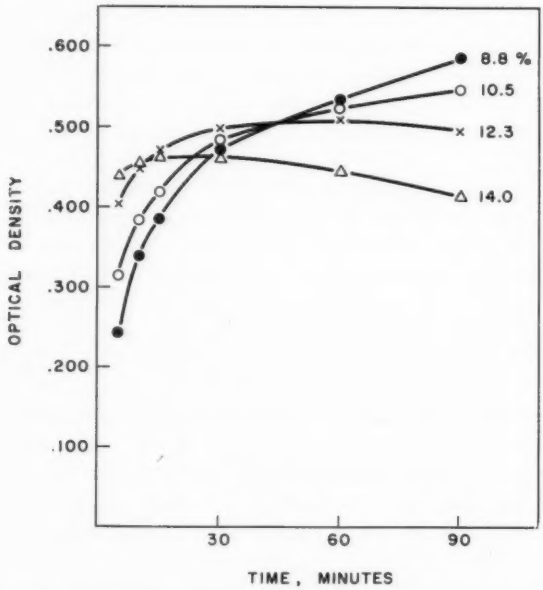

FIG. 1, A: Effect of water on sensitivity and stability. Reaction temp., 32° C.; citric acid, 200 μg/test; total volume, 11.4 ml. Pyridine, 15.8%; acetic anhydride, 70.1%; acetic acid plus water, 14.1%; per cent water is shown by the figures on the curves.

excess of either of these reagents promoted color-fading. Similar effects were observed at other reaction temperaures and with other concentrations of citric acid between 25 and 200 μg. per test. The effect of acetic anhydride could not be illustrated at temperatures below 44° C., because a significant excess could not be added without drastically affecting the pyridine and water concentrations. However, at a reaction temperature of 60° C., the effect of anhydride was similar to that of pyridine and water (Figure 1, C).

Studies were made at several reaction temperatures to determine the optimum proportion of reagents for the development of color of maximum intensity, compatible with a stability of at least 30 min. In this study, glacial acetic acid was not used except at temperatures above 44° C., where it was necessary to substitute it for part of the acetic anhydride, as high concentrations of the latter caused rapid color-fading. The optimum proportions of reagents (% volume / arithmetic total volume) are shown (Figure 2). The amounts of reagents used by Saffran and Denstedt *(10)*, assuming complete removal of water by hydrolysis of acetic anhydride, agreed with our optimum conditions at 60° C., but those used by Babad and Shtrikman *(1)* deviated markedly.

A comparison of results obtained at optimum reagent proportions for various temperatures showed that maximum intensity and stability were obtained at reaction temperatures between 17 and about 35° C. (Figure 3); sensitivity

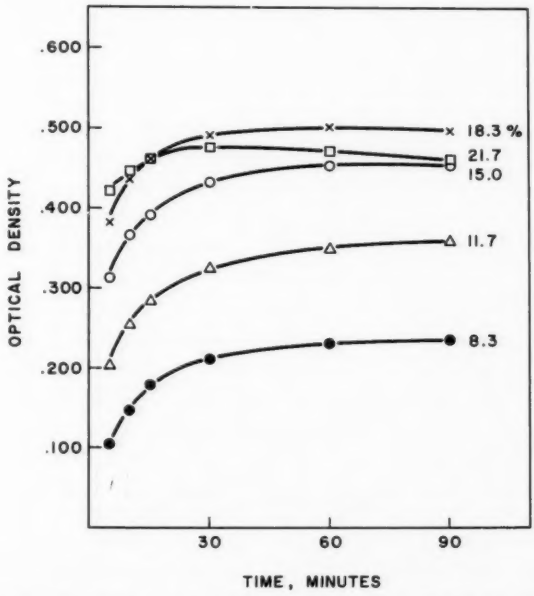

FIG. 1, B: Effect of pyridine on sensitivity and stability. Reaction temp., 32° C.; citric acid, 200 μg/test; total volume, 12.0 ml. Water, 11.7%; acetic anhydride, 66.6%; acetic acid plus pyridine, 21.7%; per cent pyridine is shown by the figures on the curves.

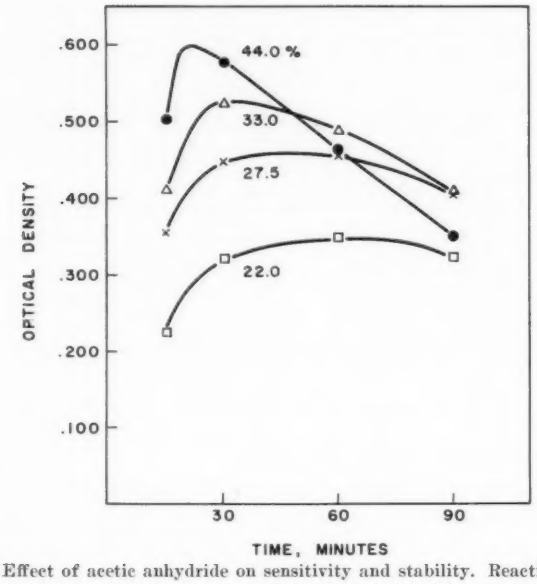

FIG. 1, C: Effect of acetic anhydride on sensitivity and stability. Reaction temp., 60° C.; citric acid, 200 μg/test; total volume, 9.1 ml. Water, 1.1%; pyridine, 11.0%; acetic acid plus acetic anhydride, 87.9%; per cent acetic anhydride is shown by figures on the curves.

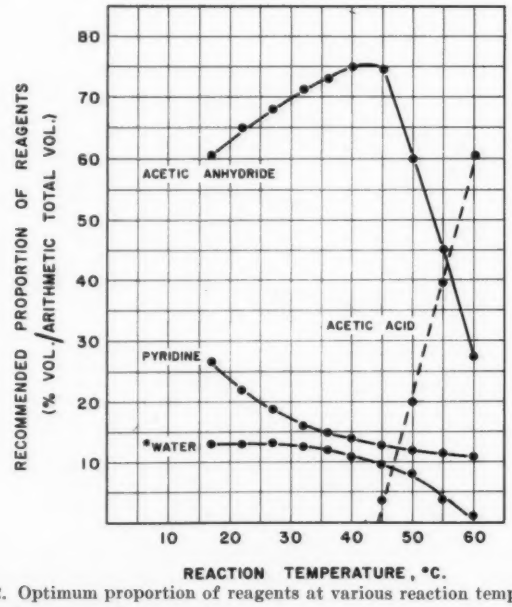

FIG. 2. Optimum proportion of reagents at various reaction tempeatures.
* Includes water of the sample.

and stability decreased slightly at 40° C. and markedly at higher temperatures. The present technique at 32° C. was more sensitive than either the Saffran and Denstedt *(10)* or the Babad and Shtrikman *(1)* procedures (Figure 4). Reproducibility of the method at 32° C. was assessed by analyzing standard solutions of citric acid 14 times during a 3-wk. period; the results showed a coefficient of variability of 0.37%, compared to 1.0% obtained in a parallel study with the Saffran and Denstedt method.

The optimum conditions for use with the present technique were determined at constant temperature throughout the holding period. However, for routine

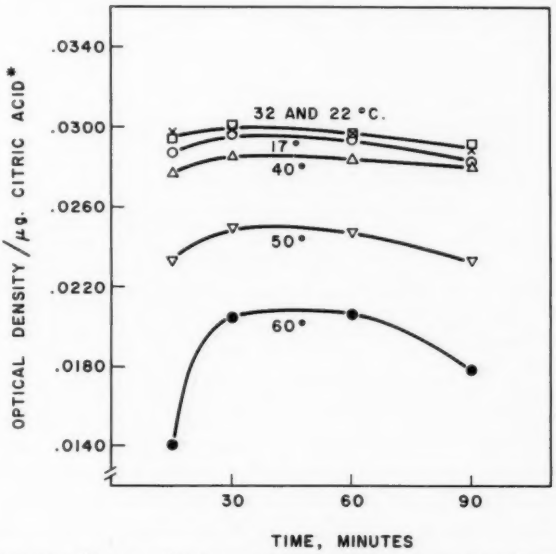

FIG. 3. Effect of various reaction temperatures, using optimum reagent concentrations, on color intensity and stability. (Average of results with 25, 50, 100, and 200 μg. of citric acid per test.)

* Corrected to a 1.0-ml. final volume for all temperatures. [Dividing the given value by the total volume (ml.) used in the test will give the actual sensitivity coefficient obtainable.]

analysis, it was convenient to withdraw the samples from the bath as a group after color production was complete (e.g., 30 min.), and the effect of subsequent holding in air at several temperatures was, therefore, investigated. Color stability could be maintained for 30 min. by holding the samples in air at a temperature of 3–10° C. below the bath temperature. If the gradient between bath and room temperatures is not as recommended, readings should be made within 10 min. after removing the tubes from the bath or, if more desirable, another reaction temperature can be selected. Cooling in an ice bath, as previously recommended (*1, 6, 8, 10*), results in a loss of stability and should, therefore, be avoided.

It appears probable that the changes in color intensity with time are the re-

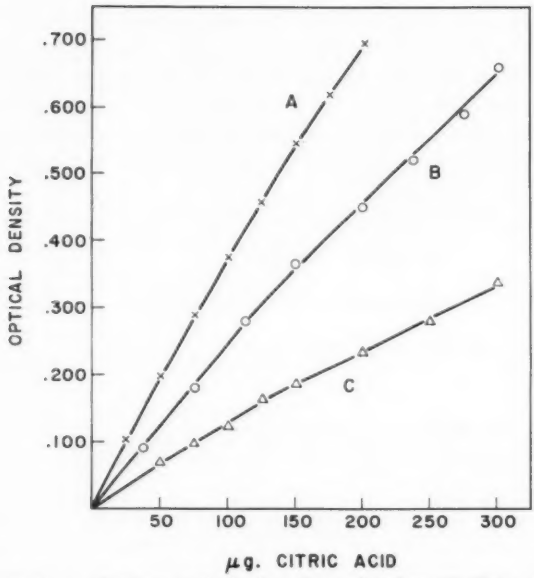

FIG. 4. Comparison of three methods for the estimation of citric acid. A: Present method, 32° C., 8.0 ml. total volume. B: Saffran and Denstedt method *(10)*, 60° C., 10.0 ml. total volume. C: Babad and Shtrikman method *(1)*, 60° C., 8.2 ml. total volume.

sult of three reactions occurring almost simultaneously, i.e., a color-forming reaction, a color-destroying reaction, and hydrolysis of acetic anhydride with water. All three reactions are affected by temperature, and by concentration of pyridine, water, and acetic anhydride. The ingredients responsible for color production are also involved in its destruction (fading) with time, especially when in excess (Figure 1, A, B, and C). Color formation is dominant initially but, as would be expected, soon decreases in rate as citric acid or one of the reagents becomes limiting; meanwhile, the rate of color-destroying reaction increases in proportion to the degree of pigmentation. When the color-forming and color-destroying reactions are occurring at equivalent rates, the color intensity remains constant for a time interval related to the over-all rate of reaction. If—at this stage of the reaction—the rate of both reactions is decreasing, the period of apparent equilibrium will be increased. Hydrolysis probably acts in this manner, reducing the rate of reaction by decreasing the concentration of water and acetic anhydride.

RECOMMENDED METHOD

Apparatus

a) A colorimeter or spectrophotometer, equipped to measure color intensity at 420 mμ (an Evelyn colorimeter was used in this work).

b) A water bath at 32 ± 0.25° C., with sufficient capacity to insure dissipation of the heat generated by the reaction. (With moderate stirring, and no

cooling coil, the bath capacity should be at least 400 ml. of water per sample).

c) Self-filling pipettes for both acetic anhydride and pyridine. Prolonged inhalation of pyridine vapor is injurious to health *(2)*.

Reagents

a) Reagent-grade acetic anhydride and pyridine.

b) Anhydrous citric acid γ obtained by heating the monohydrate at 90° C. to constant weight—about 72 hr.—was used to prepare a stock solution (50 mg/ml) which is stable for at least 1 yr. at 0° C. Standards, prepared from the stock solution, are stable for 1 mo. at 0° C.

Procedure

To a colorimeter tube, add 1.0 ml. of sample (containing from 25 to 200 μg. of citric acid), or citric acid standard, or water (for the reagent blank), followed by 1.30 ml. of pyridine, and swirl the tube briskly. After similar additions have been made to all tubes in a series, add 5.70 ml. of acetic anhydride, swirl the tube again, and immediately place it in the constant-temperature bath.[2] Color development is complete after 30 min., and the color is stable for at least another 30 min., either in the 32° C. bath or in air at a temperature of 22–29° C. Read the color intensity at 420 mμ with the blank set at 100% transmission and estimate the citric acid content of the samples by reference to the standards.

ANALYSIS OF MILK AND SERUM

Preparation of samples. Milk and serum are diluted directly to a citric acid content between 25 and 200 μg. per milliliter; whole milk is centrifuged, or filtered after dilution, to decrease the fat content. If serum contains trichloracetic acid (T.C.A.), prepare an extra blank containing an equivalent amount of T.C.A. and subtract the optical density (2-log G) of this blank from that of the samples; this will correct the samples for the increase in absorption caused by T.C.A. Homogenized milk requires a correction for the turbidity introduced by fat. To determine this correction, the recommended "Procedure" is used with a separate aliquot of the dilute milk, but 5.70 ml. of glacial acetic acid is substituted for the acetic anhydride. This allows estimation of the interfering turbidity in the absence of color. Subtract the optical density of this solution from that obtained in the actual test, and calculate the citric acid content of the test sample from the corrected value.

Possible interfering substances. Calcium chloride and monopotassium phosphate were added to standard citric acid solutions so as to introduce various proportions (from 100 to 600 μg.) of calcium and phosphorus with the 1.0-ml. aliquot. The opalescence that formed on addition of pyridine to these solutions disappeared after addition of acetic anhydride, and the intensity and stability of the color were not affected.

[2] Turbidity forms on addition of acetic anhydride to solutions containing protein, but clears during the holding period in the bath.

The effect of lactose was studied by introducing up to 50 mg. of the milk sugar with the 1.0-ml. aliquot. Color intensity was reduced by about 2% for every 10 mg. of lactose added, but since 5 mg. or less is present in suitable dilutions of milk, no correction is required.

Values obtained after application of the turbidity correction for fat (described above) agreed within 2.0% with the value for the same milk after fat separation, indicating that milk fat did not contribute to color development.

Possible interference by acids used in the preparation of milk será was investigated. The effect of hydrochloric and trichloroacetic acids on the intensity and stability of the color was studied by analyzing aliquots of standard citric acid solutions containing 5% T.C.A., or various concentrations of hydrochloric acid up to 1.0 *N*. Hydrochloric acid did not affect the intensity or stability of the color; T.C.A., although it did not affect stability, caused a slight increase in absorption at 420 mμ, but the additional color developed to the same extent in blanks and standards. In a similar manner, the effect of sodium hydroxide was studied. It was found that aliquots of NaOH of up to 0.1 *N* concentration could be tolerated.

The effect of milk proteins on the Babad and Shtrikman method *(1)* has been studied by Murthy and Whitney *(6)*, who concluded that the presence of residual protein in milk serum resulted in erroneously high citric acid values. In our experiment, the possible interference of casein was investigated with 1% solutions of casein [Van Slyke and Baker *(12)*] in 0.1 *N* NaOH, or anhydrous pyridine. For the test, full aliquots of the pyridine or NaOH solution replaced—respectively—the pyridine or water required in the normal procedure. The test solutions obtained with the NaOH preparation were clear, but a faint turbidity—for which no correction was made—was present in the pyridine samples (this casein turbidity is not encountered in direct analysis of milk). The turbidity correction described could not be applied, because glacial acetic acid dissolves the casein.

The results indicated that solutions of casein in pyridine contained chromogenic material equivalent to 1.0 μg. of citric acid per milligram of casein, whereas the NaOH solution contained 2.0 μg per milligram. Precipitating the casein from the NaOH solution with hydrochloric acid failed to reduce the amount of chromogenic material obtained on re-solution of the casein, while an approximately equivalent amount was found in the mother liquor. These results indicate that isolated casein contains little chromogenic material (even if no allowance is made for the turbidity present in the pyridine test), but that appreciable amounts may be formed during dissolution in alkali. Interference by the casein in milk should, therefore, be less than that observed with isolated casein. However, even if one assumes chromogenic material in casein equivalent to 1.0 μg. of citric acid per milligram, the error involved in the direct analysis of milk (assuming 1,700 μg. of citric acid per milliliter and 3% casein) would be 1.8%.

A study of the combined effect of all milk components, on the reaction involved in the test, was done with solutions containing various amounts of skim-milk at fixed concentrations of citric acid. These solutions, prepared by adding

known amounts of citric acid to dilutions of milk to give final concentrations of approximately 80 or 200 μg. per test, were analyzed and the results throughout agreed with the expected total, within 1.5% (Table 1).

TABLE 1

Analysis of solutions containing various amounts of skimmilk, adjusted to constant levels of citric acid

Skimmi k added (*ml. per test*)	*Citric Acid (μg. per test)*			
	Added	Determined	Expected	% Accuracy
0.120	00.0	213	.....	.....
0.105	25.5	213	211.9	100.5
0.090	51.0	213	210.8	101.0
0.075	75.8	212	208.9	101.5
0.060	101.3	208	207.8	100.1
0.045	126.8	206	206.7	99.7
0.030	152.3	205	205.6	99.7
0.015	177.0	205	203.6	100.7
0.045	00.0	80.3	.....	.....
0.030	25.5	79.5	78.8	100.9
0.015	50.3	76.5	76.9	99.5
			Average	100.4

Application. A sample of skimmilk was analyzed at six intervals during a three-day period and the results varied from 1,685 to 1,694 μg. per milliliter, with an average of 1,689 ± 4 μg. The milk was stored at 0° C., and a fresh dilution was prepared for each analysis.

The method has been applied to the analysis of skimmilk, heated skimmilk, commercial evaporated milk, instant skimmilk powder, sera prepared from the various milks by precipitation with 12% T.C.A. *(9)*, and sera prepared from skimmilk by precipitation with 6% v/v addition of 1.0 *N* hydrochloric acid, by coagulation with rennet *(5)*, and by ultrafiltration *(11)*. Determinations were made at a reaction temperature of 32° C., also at 17 and 50° C. (with the optimum reagent proportions given in Figure 2 and reading the samples within 10 min. after removal from the bath). Values obtained for the sera were multiplied by 0.97 or 0.94 to correct for the volume change caused by removal of protein, or protein plus fat, respectively.

The results obtained (Table 2) at the three temperatures agreed within 2.5%, attesting to the degree of accuracy obtainable at various reaction temperatures. The citric acid content of each serum was lower than that of the corresponding milk by approximately 10% (15.1% for the ultrafiltrate). The apparent amount of chromogenic material measured in isolated casein would account for only about 2% of this difference, while the agreement between results of sera prepared with T.C.A. and either HCl or rennet indicates that serum proteins do not interfere.

Throughout our investigations, there has been no evidence to suggest that results of direct analysis of milk are erroneously high. Of the milk components, only fat has been found to interfere at levels likely to be encountered in the course of analysis. Attempts to detect interference of proteins have indicated that they do not interfere significantly. Ultrafiltration invariably yields a low

TABLE 2

Citric Acid content of skimmilk, evaporated milk, and milk powder, and their sera, determined at various reaction temperatures

	Citric Acid (μg/ml)		
	17° C.	32° C.	50° C.
Skimmilk, untreated	2,198	2,182	2,142
Skimmilk, heated[a]	2,205	2,251	2,226
Skimmilk, T.C.A. serum	2,088	2,024	2,062
Skimmilk, HCl serum	2,056	2,042	2,047
Skimmilk, Rennet serum	2,048	1,983	2,018
Skimmilk, Ultrafiltrate	1,882	1,853	1,943
Evaporated milk[b]	2,023	1,992	2,101
Evaporated milk, T.C.A. serum	1,954	1,915	1,953
Skimmilk powder[c]	1,891	1,890	1,881
Skimmilk powder, T.C.A. serum	1,695	1,687	1,700

[a] Skimmilk, heated at 180° F. for 15 min.
[b] Commercial evaporated whole milk, reconstituted 1:2 with water.
[c] Commercial instant skimmilk powder, reconstituted to 9% solids with water.

value for serum citric acid, a result which can be ascribed to casein:citrate association as reported by Eilers and Jense *(3)* and White and Davies *(13)*. The intermediate values obtained with acid and rennet sera can be attributed to adsorption of citric acid by the precipitated protein, as suggested by Pucher *et al. (7)*. We are, therefore, convinced that the higher values obtained by direct analysis of milk represent its true citric acid content within 2.5%.

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EFFECT OF VARIATIONS IN FAT AND TEMPERATURE ON THE SURFACE TENSION OF VARIOUS MILKS[1, 2]

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SUMMARY

Measurements of the surface tension of skimmilk, whole milk, and homogenized milk were made at 60, 81, and 102° F. with a du Noüy tensiometer, to obtain data for use in the calibration of lactometers. The results are presented graphically. A consistent difference was apparent between the surface tensions of the three milks, with an increasing divergence occurring with a rise in temperature. The difference between the surface tension of skimmilk and whole milk was about 50% greater at 102 than at 60° F. The surface tension of homogenized milk was intermediate to that of skimmilk and whole milk. The change in the surface tension with temperature for all milks was most marked between 60 and 80° F. The surface tension of whole milk was not affected significantly by fat in excess of about 4%.

The present work on the surface tension of milk was done in connection with research on the calibration of lactometers to be used at the temperature of 102° F. *(18, 19)*. It has been the practice to verify the calibration of lactometers by reading them in sulfuric acid solutions of known specific gravity. The calculations involved in correcting the readings in acid on a basis of 60/60° F. to readings in milk on a basis of 102/102° are complicated, and the values obtained are made uncertain by the lack of information about the surface tension of milk at the different temperatures. Inaccurate corrections for surface tension in the calibration of lactometers can result in errors as large as 0.5 degree Quevenne *(9)*. A search of the literature revealed that information on the surface tension of various milks over the temperature range of 60–102° F. was meager, and entirely inadequate for the purposes of the study. Also, it has been pointed out that the calibration of lactometers should be verified by observations made on skimmilk and homogenized milk, in which any difficulties due to the rising of the fat can be avoided *(19)*. Therefore, a study of these milks was included in this investigation.

EXPERIMENTAL PROCEDURE

The experimental work was carried out with a du Noüy tensiometer which had been accurately calibrated *(4, 13)*, and which was equipped with a ring which had a mean circumference of 6 cm. Pyrex vessels with an inside diameter of 67 mm. and a depth of 49 mm. were used to hold the 60-ml. milk samples, and were enclosed in an insulated container for the measurements. The milk was protected during the determinations by a cover with apertures for passage of the wire sup-

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[1] This work was done with funds from the Agricultural Marketing Service.

[2] Presented at the Fifty-first Annual Meeting, American Dairy Science Association, Storrs, Connecticut, June 19-21, 1956.

porting the ring and an accurately calibrated thermometer. Milk temperatures were held constant at 60° F. by precooling the milk and containers and carrying out the determinations in a constant-temperature room at 68° F. Temperatures of 81 and 102° F. were held constant by the use of a small 10-watt heating coil embedded in the insulated container. The ambient temperature for measurements at 102 and 81° F. was approximately 80° F.

Determinations of surface tension were made on 72 samples of various milks, of which 27 were raw skimmilk, 23 were pasteurized, homogenized milk from five sources (four commercial), and 22 were raw, whole milk. The number of samples of each milk tested at the temperatures of 60, 81, and 102° F. were, respectively: skimmilk, 7, 4, and 16; homogenized milk, 8, 7, and 8, and whole milk, 5, 4, and 13. Either two or three readings were made on each sample and the results were averaged for each determination. The temperatures of main interest were 60 and 102° F. Some values at the midway point of 81° were obtained, in order to plot curves. Half of the skimmilk samples were a few hours old and half were about one day old. The age of the whole milk samples varied from a few hours to about 12 hr. The age of the commercial, homogenized milk was estimated to be about two days.

Fat determinations were made on all samples of milk, with the exception of two of skimmilk. The DPS detergent method *(14)* was used for milk and the American Association[3] modified Babcock method *(7)* for skimmilk. The skimmilk samples ranged in fat content from 0.03 to 0.08%, with one of 0.22%. The commercial, homogenized milks ranged from 3.4 to 3.7% fat, with two laboratory homogenized samples of 2.5 and 6.2% fat. The whole milk samples varied in fat content from 3.1 to 7.7%.

RESULTS

The surface tension, as calculated from least-squares regression equations, and their standard errors of estimate for the different milks over the temperature range of 60–102° F., are shown (Figure 1). The results show that there was a consistent difference in the surface tensions of skimmilk, whole milk, and homogenized milk, with an increasing divergence apparent with a rise in temperature. The surface tension of the homogenized milk fell about midway between that of the other milks. The fact that there was an increase of about 50% in the difference in surface tension between skimmilk and whole milk when the temperature was raised from 60 to 102° is of importance in the calibration of lactometers at the higher temperature. The rate of change in the surface tension with increased temperature for all milks was greatest between 60 and 80° F.

The surface tension of skimmilk and whole milk containing various percentages of fat is shown (Figure 2). The points for skimmilk are average values, whereas those for whole milk are individual values. The data show a definite difference between the surface tension of skimmilk and that of whole milk. It is also evident that surface tension is not greatly affected by a fat content in

[3] American Association of Creamery Butter Manufacturers.

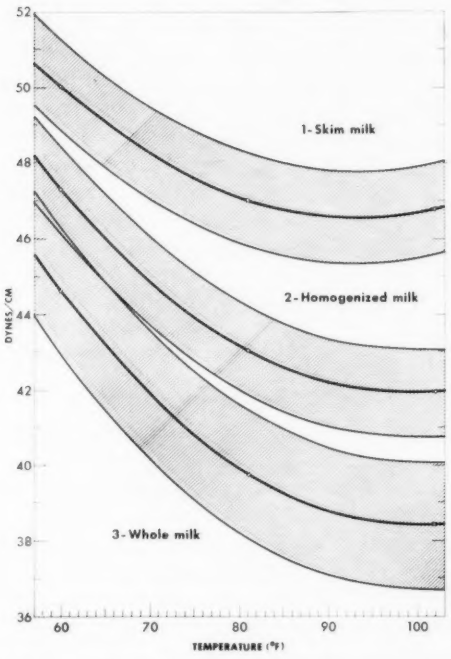

FIG. 1. Variation of the surface tension of milks with temperature. Shaded areas indicate the standard errors of estimate.

Y = Surface tension in dynes/cm. X = Temperature in ° F.

1. $Y = 74.25 - 0.5941X + 0.003185X^2$ Standard error of estimate = ± 1.22
2. $Y = 76.73 - 0.7054X + 0.003574X^2$ Standard error of estimate = ± 1.16
3. $Y = 77.92 - 0.7953X + 0.004004X^2$ Standard error of estimate = ± 1.70

excess of 4%. This is in general agreement with the conclusions of Dunkley *(6)*, who worked with reconstituted milk of varying fat content.

The values for the surface tension of skimmilk and whole milk are in good agreement with the results of Dunkley *(6)*, Hetrick and Tracy *(10)*, and Sharp and Krukovsky *(15)*. However, the values are considerably lower than other static surface tension readings on milk reported in the literature, which include those by Cardoso and Wancolle *(3)*, Kopaczewski *(11)*, Nutti *(12)*, Whitnah *et al.* *(20)*, and Velu and Belle *(17)*.

DISCUSSION

The lack of agreement in the published results is not surprising when the influence of the various circumstances involved is considered. Some of the factors are variations in the composition of milks from widely scattered sources, and the age and prior treatment of the samples before measurement. The different types of apparatus, and the methods employed, also affect the precision of the measure-

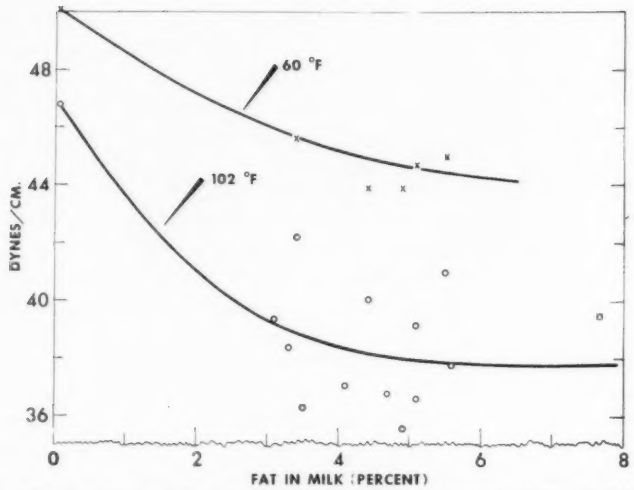

FIG. 2. Effect of fat variations in milk upon the surface tension at 60 and 102° F.

ments. It seems apparent that in some instances a correction factor as suggested by Harkins and Jordan *(8)* and Zuidema and Waters *(21)* was not applied to the results obtained by the ring method, which would result in a positive error of almost 10% in the surface tension when a 6-cm. ring is used. The error would be considerably larger for a 4-cm. ring.

The surface tension of the homogenized milk averaged about three dynes higher than that of the whole milk over the temperature range of 60–102° F. Doan *(5)* and Trout *et al. (16)* reported that the surface tension of milk was increased by homogenization if the milk was pasteurized before processing. Doan attributed the increase to the greater protein adsorption by the fat resulting from the increase in the fat surface.

It is well known that the development of rancidity in milk markedly lowers the surface tension. The homogenization of raw milk sometimes results in a lowering of the surface tension and this effect may be due to lipase action. This would not be a significant factor in the surface tension studies of pasteurized, homogenized milk reported here.

Some workers have reported that milk on aging undergoes a small decrease in surface tension which appears to be due to solidification of the fat or to lipase action. However, experiments in this study showed a decrease of only about one dyne for herd milk and no change for commercial homogenized milk in the surface tension at 81° F., when the milks were held at 32° F. for two and three days, respectively.

Burri and Nussbaumer *(2)* and Bauer *(1)* noted a decrease in the surface tension of milk with time, and attributed it to the solidification of the fat, because the surface tension returned to its former value when the milk was heated for 30 min. at 50° C.

Whitnah *et al. (20)* also found that the condition of the milk fat affected the surface tension readings. They reported a decrease in the surface tension of commercial homogenized milk held for seven days at about 5° C. over the same milk held for one day and then warmed to 15, 25, and 30°. The decrease was 5.5, 2.9, and 1.3 dynes, respectively. However, when the milk was warmed to 40, 50, and 60° C., the changes in surface tension were negligible. This information indicates that the surface tension of milk tends to be more uniform, regardless of age, when milk is heated to a temperature at which the fat is in a liquid condition, which is a point in favor of using a heating method for lactometer readings.

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METHYL KETONES IN EVAPORATED MILK [1]

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SUMMARY

Acetone, pentanone-2, and heptanone-2 were identified as the principal volatile carbonyl compounds in low-temperature, reduced-pressure distillates from commercial evaporated milk. Of these, only heptanone-2 is considered to be of possible flavor significance. Quantitative studies with simplified milk systems and six commercial brands of evaporated milk indicated that the C_5 and C_7 ketones are generated by action of heat on the fat phase. Acetone was detected in unheated milk, but heating enhanced its content. The findings do not preclude the presence of a larger homologous series of methyl ketones in the product. The conditions of distillation (45° C., 25 mm. Hg pressure for 4 hr.) did not appear to volatilize the main caramel flavor components of evaporated milk to any great extent.

When milk is subjected to sterilizing heat treatments a flavor, perhaps best described as caramelized, develops. This flavor intensifies during storage of the sterilized product at room temperature. In general, these phenomena are typified in evaporated milk. Little seems to be known regarding the identity of compounds contributing to flavor in this product. To throw further light on the matter, a study of the volatile carbonyl compounds in evaporated milk was undertaken.

EXPERIMENTAL PROCEDURE

Collection and detection of carbonyl compounds in evaporated milk distillates. The contents of from 20 to 24 cans (14.5 oz.) of Carnation evaporated milk were low-temperature, reduced-pressure distilled, using a technique similar to that of Day *et al. (2)*. Only three traps were used after the cold water condenser, a wet ice trap, followed by two dry ice–ethanol traps. About one liter of distillate was collected in the wet ice trap after 4 hr. of distilling at 45° C. and 25 mm. Hg pressure, and only small quantities (up to 20 ml.) collected in the dry ice–ethanol traps. Each trap was observed for odor, after which the distillates were combined. A summary of the observations on the odor of the distillation flask and individual traps is as follows:

Distillation flask: Caramel, similar to original sample.
Wet Ice Trap: Cooked cabbage, malty, cheesy, heptanone-2, methyl sulfide, ketones.
First Dry Ice Trap: Heptanone-2, cooked cabbage.
Second Dry Ice Trap: Heptanone-2, cooked cabbage, malty, rubber-like.

Since the odor of the distillate suggested the presence of methyl ketones, the distillate was treated with 10 ml. of 1% DNPhydrazine[3] in 30% sulfuric acid

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[3] 2,4-Dinitrophenyl is abbreviated DNP throughout.

and held overnight. One hundred-milliliter quantities of the treated distillate were extracted with three 100-ml. quantities of carbonyl-free petroleum ether (b.p. 30–60° C.)[4] in a one-liter volumetric flask on a Burrell automatic shaker for 6 hr. The extracts were combined, washed with 50 ml. of water, and dried over anhydrous sodium sulfate. About 10 ml. of this petroleum ether extract was reduced to 1-ml. volume and the concentrated extract paper-chromatographed by the method of Huelin *(4)*. In addition, reference spots of acetone, pentanone-2, and heptanone-2 derivatives were run on all chromatograms. Figure 1 répresents a

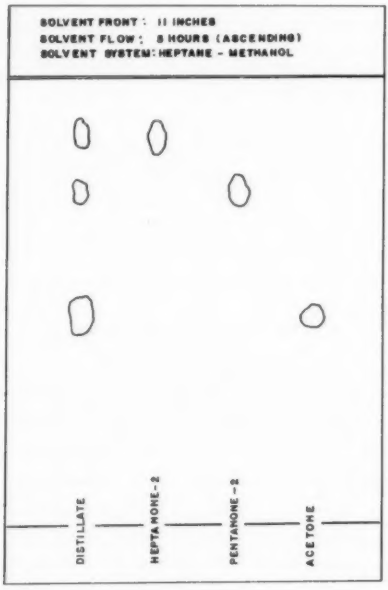

FIG. 1. Reproduction of a paper chromatogram of 2,4-dinitrophenylhydrazones from evaporated milk distillate and from certain known ketones.

chromatogram of the DNPhydrazones from the distillate of evaporated milk. The extract resolved into three principal fractions, corresponding to the R_f values of acetone, pentanone-2, and heptanone-2 DNPhydrazones.

Further identifying information of the methyl ketones was obtained through ultraviolet spectral analysis. The resolved bands that were observed on a developed chromatogram when the distillate extract was streaked along the base-

[4] Butanone and lesser amounts of other carbonyls have been observed as consistent trace components of all low-boiling petroleum ether at this laboratory. These were removed by successively treating with DNPhydrazine in 30% sulfuric acid, shaking the mixture for 8 hr., redistilling the solvent layer, and repeating this process five times.

line were cut from the paper and extracted with from 5 to 10 ml. of 95% ethanol. The light absorption spectrum of each solution was determined in a Beckman D.U. Spectrophotometer. An absorption maximum was observed at 362 mμ for each band. These values are in agreement with values for the derivatives of saturated aliphatic ketones reported in the literature *(1)*.

Isolation and identification of ketone derivatives. Paper chromatographic methods are essentially analytical, rather than preparative, and do not lend themselves to the fractionation of of sufficiently large amounts for melting point determination. For this reason, column chromatography using the Huelin solvent system was employed. Columns were prepared with Whatman ashless cellulose powder (coarse grade) which had been previously washed with large volumes of ethanol and dried at 75° C. and reduced pressure. Equilibration was effected by sealing ten parts of cellulose and three parts of the methanol-rich phase in a one-liter Erlemeyer, shaking very vigorously until homogeneity was obtained, and allowing it to stand overnight. The equilibrated cellulose was packed into a 2.5- by 30-cm. chromatographic column by compacting small amounts with a rubber stopper attached to a wooden dowel. The column then was filled with the heptane phase and allowed to drain. The solution of DNPhydrazones extracted from the distillate with petroleum ether was evaporated to dryness. The residue was dissolved in a minimum of heptane phase (about 2 ml.) and added to the top of the column. The hydrazones were washed into the partition system with several 10-ml. portions of heptane phase. Heptane phase then was added to the reservoir and the column allowed to develop. The mixture of DNPhydrazones separated into three bands after 3 hr., but the bands of heptanone-2 and pentanone-2 were faint and often difficult to observe. When these fractions were collected, paper chromatograms indicated in many cases that fractions were not pure but were contaminated by adjacent bands. Some degree of purity was obtained when 10-ml. quantities were collected from the time the eluate started to turn yellow until the last band was eluted. The fractions were paper-chromatographed and similar fractions were combined. Fractions containing more than one compound were discarded.

The solvent was removed from the combined fractions with the aid of heat (water bath at 70° C.) and reduced pressure. The residues were dissolved in a minimum of warm petroleum ether, and placed in a refrigerator to promote crystallization, which usually occurred within two to three days. Crystals were washed with water and air-dried for several hours before determination of melting points. Melting points were determined on a Fisher micro-melting point apparatus. The acetone fraction melted at 124 to 125° C. and a mixed melting point with authentic acetone derivative (127°) was from 124 to 126° C. The pentanone-2 fraction gave a melting point of 142–143° C. after recrystallization by sublimation. These crystals and an authentic pentanone-2 derivative (m.p. 142.5–143.5° C.) gave a mixed melting point of 142–143° C. The melting point of 69–70° C. for the heptanone-2 fraction was obtained only after many attempts yielded melting points in the 54–58° C. range. A mixed melting point run with an authentic heptanone-2 derivative (72° C.) gave a melting range of 68–70° C.

Infrared spectra from crystals of the unknown and authentic heptanone-2 derivative, obtained on KBr pellets using a Perkin-Elmer Model 21 Infrared Spectrophotometer, were essentially identical.

Quantitative measurements of methyl ketones in evaporated milk and heated milks. Quantitative measurements were made on raw milk and sterilized milk samples, in addition to various brands of locally procured evaporated milk. The milk samples were obtained from the University Creamery as the raw milk was being separated. A raw milk sample was retained as a control and samples of whole milk, skimmilk, and cream were sealed in 14.5-oz. cans and sterilized in a Fort Wayne Sterilizer for 15 min. at 116° C.

The procedure employed in obtaining the crude DNPhydrazones was generally the same as the procedure outlined above. Four cans (14.5 oz.) of sample were low pressure–distilled directly into 60 ml. of 0.2% DNPhydrazine in 30% sulfuric acid. An aliquot of 200–300 ml. of each distillate was extracted with petroleum ether, and one-half of the extract was paper-chromatographed. (It is often necessary to streak more than one paper to avoid overloading.) The hydrazone bands of heptanone-2, pentanone-2, and acetone on the developed chromatograms were cut from the paper and extracted with ethanol. The optical density of these solutions was measured at a wavelength of 352 mμ. To determine the quantities of heptanone-2, pentanone-2, and acetone present in the sample, the following formula was used:

$$\text{Concentration of ketone in mg/liter in sample} = \frac{D \times W_1 \times 1{,}000}{k \times W_2 \times A \times B}$$

where D = optical density (1-cm. cell)

W_1 = molecular weight of ketone

k = extinction coefficient[5]

W_2 = molecular weight of ketone hydrazone

A = dilution factor determined from the volume of ethanol used to extract the band; that is, if the paper was extracted with 10 ml. of ethanol the factor would be $\frac{(1{,}000)}{(10)} = 100$ for the concentration as mg/liter.

B = dilution factor representing the size of the sample used; for example, if the quantity on the paper represented one can (one 14.5-oz. can equals 400 ml.) the factor would be $\frac{(400)}{(1{,}000)} = 0.4$ to convert to one liter of sample.

The amounts of distillate from, and ketones present in, evaporated milk (seven brands) and heated canned milk fractions are presented (Tables 1 and 2).

Determination of flavor threshold concentration. The detection and determination of the amounts of heptanone-2 and pentanone-2 in evaporated milk led to the question of whether the quantities present are of flavor significance. Conse-

[5] The extinction coefficient of each ketone hydrazone was determined from standard curves.

TABLE 1

Methyl ketones in evaporated milk

Brand	Distillate	Concentration of ketone in mg/liter[a]		
		Heptanone-2	Pentanone-2	Acetone
	(ml.)			
A	700	0.370	0.295	0.895
B	800	0.338	0.214	0.852
C	570	0.220	0.071	0.374
D	750	0.110	0.150	1.25
E	760	0.463	0.358	1.34
F	750	0.625	0.360	1.03

[a] Corrected for recovery of 40% heptanone-2 and 25% pentanone-2. (See text.)

TABLE 2

Methyl ketones in raw and heated[a] milks

Sample	Distillate	Concentration of ketone in mg/liter[b]	
		Heptanone-2	Pentanone-2
	(ml.)		
Raw whole milk	(1) 580	None	None
	(2) 650	None	None
Heated whole milk	(1) 640	0.288	0.100
	(2) 730	0.255	0.174
Heated skimmilk	(1) 640	None	0.005
	(2) 600	None	0.009
Heated cream	(1) 520	3.25	1.10
	(2) 560	2.60	0.700

[a] In sealed 14.5-oz. cans at 116° C. for 15 min.
[b] See footnote, Table 1.

quently, threshold values were determined by the spray technique *(8)*. The evaluations were made by preparing various levels of the individual compounds in distilled water and determining the average concentration at which five taste observers could detect the compound 50% of the time. The threshold value obtained for heptanone-2 was 0.6 mg/liter (or p.p.m.) and for pentanone-2 was 7.0 mg/liter.

Quantitative recovery analysis of heptanone-2 and pentanone-2. To determine the extent to which volatile compounds are recovered by the low-temperature, reduced-pressure distillation method, the following experiments were undertaken: To 1.6 liter of fresh, raw whole milk was added 40 ml. of a 33 mg/liter solution of heptanone-2 and 16 ml. of an 8.1 mg/liter solution of pentanone-2. The milk was then low pressure–distilled into 60 ml. of 2.5% DNPhydrazine in 30% sulfuric acid. The distillate was extracted with carbonyl-free petroleum ether and the amounts of heptanone-2 and pentanone-2 in the extracts determined as previously described. The average amounts of ketones recovered from two distillations, when compared to the amounts added to the original milk sample, indicated that 40% of the heptanone-2 and 25% of the pentanone-2 were recovered. The rather low order of recovery for the two ketones suggested that some further evaluation should be made to determine whether lack of volatility of the ketones, under conditions of distillation, or incompleteness of recovery by the DNP reagent procedure, was responsible for the discrepancy. An aqueous solution containing

measured amounts of the two ketones was reacted with the hydrazine reagent under comparable conditions, and the resulting hydrazones were quantitatively analyzed as previously described. The data indicated that approximately 77% of the heptanone-2 and 36% of the pentanone-2 are recovered in aqueous solution by analysis with DNPhydrazine. This information, by comparison with the data on the recovery through distillation and reaction with DNPhydrazine, indicates that the distillation procedure delivers about 70% of the pentanone-2 and 50% of the heptanone-2. These values might be expected on the basis of the respective boiling points, 102 and 152° C., for the two compounds. Thus, the poor recovery of pentatone-2 is attributed largely to the analysis with DNPhydrazine; whereas, this was of secondary importance to lack of volatilization in the case of heptanone-2.

DISCUSSION

Methyl ketones are known to be of significance in the flavor of Blue cheese *(5, 7)*, and it has been speculated previously that they may represent flavor defects in other dairy products *(3)*. Sufficient evidence was accumulated in this study to establish the presence of heptanone-2, pentanone-2, and acetone in evaporated milk. The detection of only these ketones in the medium does not preclude the presence of higher molecular weight members of the homologous series to which those identified belong. It has been shown by the recovery data that the temperature and pressure relationship used in the distillation may remove only about one-half of the heptanone-2 actually present. The recovery of higher boiling members of this homologous series no doubt would be progressively less and eventually nil. The poor recovery of ketones by the quantitative analytical method based on DNPhydrazine suggests that such analysis represents a substantial research problem in itself. However, for the exploratory purposes of determining origin and possible flavor significance of these ketones in this study, the method as used appears adequate.

The fact that heptanone-2 and pentanone-2 were found in heated whole milk but were undetectable in raw milk (Table 2) leads to the conclusion that these compounds are heat-generated. The quantities of them in heated cream was observed to be many times greater than in heated skimmilk, which leaves little doubt that they arise from the lipid phase. Although quantitative analyses were not made on acetone in raw and heated milk fractions, judging from the density of spots for its hydrazine on paper chromatograms heat treatment enhanced acetone content, and the greatest increase seemed evident by heating cream. The specific nature of the precursors of these ketones in the milk lipids is not known. However, β-keto-acids are perhaps the most common origin of methyl ketones in biological systems, the conversion being a matter of decarboxylation. β-keto-acids might occur in milk either as degradation products of fatty acids by β-oxidation or as intermediates in the synthesis of fatty acids from acetate by the mammary gland. Deterioration of the milk lipids through attack by atmospheric oxygen may offer yet another explanation for the origin of the ketones. Such a mechanism

is considered to be a rather remote possibility in heated cream or evaporated milk, media which contain a wealth of reducing substances.

Of the compounds identified, only heptanone-2 (threshold 0.6 p.p.m.) perhaps is of flavor significance. It is definitely more potent flavorwise than pentanone-2 (threshold 7.0 p.p.m.) and is found in larger quantities. The quantities found in evaporated milk (Table 1) are close to the threshold range of possible organoleptic detection. Acetone was omitted from consideration of flavor significance since previous work *(8)* showed its threshold to be at least 40 p.p.m.

In an earlier study *(6)*, acetaldehyde and furfural were demonstrated as the principal volatile carbonyl compounds produced by heat treatment of skimmilk. These two compounds were not evident in the present investigation. The use of petroleum ether as an extracting solvent, in which the derivatives of these compounds are poorly soluble, may account for this difference. It is also possible that these aldehydes are produced to a much more limited extent by heat treatment than are the ketones. Acetaldehyde may have contributed some malty character to the odor of dry ice–ethanol traps in the distillation of evaporated milk; however, these traps had an odor mainly resembling those in the distillation of radiated skimmilk *(2)* and suggesting the presence of methyl sulfide, methyl mercaptan, and methyl disulfide.

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EFFECTS OF HIGH LEVELS OF VITAMIN D_2 FED PREPARTUM ON THE INCIDENCE OF PARTURIENT PARESIS IN DAIRY CATTLE

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SUMMARY

This investigation was conducted to determine the value of high-potency irradiated ergosterol (vitamin D_2) as a preventive for parturient paresis. The investigation involved 32 herds of Jersey cattle and data on over 500 individual parturitions. The daily feeding of 30 million U.S.P. units of vitamin D_2 markedly reduced the incidence of parturient paresis in multiparous Jersey cows. Data obtained show that the incidence in previous-history animals following treatment was 23.07%; whereas, the incidence among control animals was 61.29%. Data obtained show that the incidence in treatment animals without previous histories was 6.45%; whereas, the incidence among control animals was 22.58%. The effectiveness of various prepartal levels of vitamin D_2 on the incidence of parturient paresis was determined. In this investigation, prepartal levels affording the greatest protection for animals with and without a previous history were within a range of 60 to 75 million U.S.P. units, which is the equivalent of from two to three days prepartal feeding at the daily rate of 30 million U.S.P. units. An unusually high incidence of parturient paresis was found in previous-history animals completing the prescribed treatment period one or more days prior to parturition. Presumptive but not conclusive evidence indicating the possible suppression of parathyroid activity in these animals is suggested. Another phase of this investigation entailed the measurement of the changes in blood serum calcium and phosphorus levels in animals receiving the preventive treatment. The prepartal and postpartal changes in blood serum calcium and phosphorus were variable and, because of the limited data available, it was difficult to describe any definite trends.

A large number of investigators have shown that a serum hypocalcemia and hypophosphatemia are associated with parturient paresis. Prophylactic techniques used in the prevention of parturient paresis function in maintaining blood calcium levels by three methods: increasing the intestinal absorption of calcium, increasing the mobilization of calcium deposited in skeletal reserves, and increasing the urinary excretion of phosphate. Recently, two techniques for accomplishing this have been advanced.

Boda and Cole *(2)* reduced the incidence of parturient paresis by feeding rations low in calcium and high in phosphorus, beginning approximately 1 mo. prior to the expected date of parturition. Their data indicate that this technique has proved effective in reducing the incidence of parturient paresis; however, this method involves the use of rations which require ingredients not normally fed to dairy cattle.

A second technique, which appears to be a more practical approach to parturient paresis prevention, involves the oral administration of massive doses of vitamin D.

Experimental evidence has shown that vitamin D plays a part in calcium and phosphorus metabolism by exerting a direct effect on the mineralization of

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bone *(1)* and on the absorption of calcium for the gastrointestinal tract *(5, 7, 9, 12, 17, 18, 22)*.

Greig *(10)* and, later, Sjollema *(21)* were the first to suggest that vitamin D might prove beneficial in the prevention of parturient paresis. In their earlier studies, Hibbs *et al. (13, 14)* and Hibbs, Pounden, and Krauss *(16)* obtained increases in the level of blood calcium by the oral administration of one to five million U.S.P. units of vitamin D, fed daily beginning 2 to 4 wk. prepartum. However, they were unsuccessful in reducing the incidence of parturient paresis.

In more recent experiments, Hibbs and Pounden *(15)* reported that 30 million U.S.P. units of vitamin D fed three to seven days prepartum and one day postpartum furnished an effective procedure for the prevention of parturient paresis by preventing the normal fall in blood serum calcium and phosphorus during the critical 18–24 hr. postpartal period. Because of the variation in gestation length, some difficulty was encountered in predicting the exact time of calving.

The object of this study was to develop a practical and effective short-time prepartal feeding program, using the physical changes associated with impending parturition as a guide.

MATERIAL AND METHODS

In October, 1956, studies were initiated to determine the value of high-potency irradiated ergosterol as a preventive for parturient paresis. This study was carried on in 32 different herds of the Jersey breed and includes data from over 500 individual parturitions.

All animals with at least two previous parturitions were assigned to one of the four experimental groups. Groups I and II included only animals with a previous history of parturient paresis. Groups III and IV were selected at random and included only animals with no previous history for parturient paresis.

The preventive feeding program used was as follows: Beginning approximately three days before the expected date of parturition and continuing until two days postpartum, as many of the animals as possible, in the treatment groups, received 30 million U.S.P. units of vitamin D_2 in their grain ration daily. In the event parturition did not occur after five days, the feeding period was lengthened to a maximum of seven days. Animals completing the prescribed treatment period one or more days before parturition were reassigned to the noncontinuous group.

At each farm, 30 million U.S.P. units of vitamin D_2 were added to 4 lb. of the grain ration. The above was mixed in a portable batch mixer, placed in paper bags, and sealed. Two pounds of the dry ration containing the vitamin D_2 supplement were fed twice daily during the treatment period. Within each herd all animals were fed the same roughage and concentrate mixture. Clinical cases of parturient paresis were recorded, based on the diagnosis of veterinarians and recovery following calcium therapy.

A second phase of this investigation was conducted with the dairy herd

operated by the Department of Animal Science of the Maine Agricultural Experiment Station. The experimental procedure followed was identical with that described, with the following exceptions: *(a)* breeds of dairy cattle, other than the Jersey breed, having a previous history of parturient paresis, were used in the treatment groups, *(b)* in general, consumption of the preventive ration was quite satisfactory; however, several of the experimental animals, known to be fastidious eaters, did refuse grain supplemented with ergosterol. These same animals also refused grain without ergosterol. In these special instances, vitamin D_2 was administered in gelatin capsules with a balling gun and *(c)* blood samples from the jugular vein were taken prior to the initial feeding, approximately 36 hr. after the initial feeding, within 12 hr. prepartum, within 12 hr. postpartum, and approximately 24 and 48 hr. postpartum. Blood serum phosphate was determined by the method of Fiske and Subbarow *(8)*. The Roe and Kahn *(20)* method was used to determine blood serum calcium. Anticoagulants were not used. The significant differences between percentages, within the same frequency distribution, were tested using the method described by Pearson and Bennett *(19)*.

RESULTS AND DISCUSSION

Experimental evidence has indicated that the Jersey breed is more susceptible to parturient paresis than cows of the same age group in other breeds *(11, 13)*. Because of the existing evidence, this investigation entailed the almost exclusive use of Jersey cattle. An indication of the high incidence of parturient paresis in the herds studied can be seen in the controls without previous histories (Table 1). Although the numbers used were small in comparison to other experimental groups, control animals with previous histories demonstrated a marked susceptibility to parturient paresis at subsequent parturitions (Table 1). This was previously reported by Hibbs and Pounden *(15)*.

The daily feeding of 30 million U.S.P. units of vitamin D_2 markedly reduced the incidence of parturient paresis in multiparous Jersey cattle. Data obtained show that the incidence in previous-history animals following treatment was 23.07%; whereas, the incidence among control animals was 61.29%. A reduction in per cent incidence of approximately the same magnitude was found in treatment animals without previous histories when compared to controls. Data obtained show that the incidence in this group was 6.45%; whereas, the incidence among control animals was 22.58%.

The literature is devoid of specific data relating various prepartal levels

TABLE 1

Effect of feeding 30 million U. S. P. units of vitamin D_2 per day on the incidence of parturient paresis

	With previous history		Without previous history	
	Group I treatment	Group II control	Group III treatment	Group IV control
Total cows	130	31	155	155
Per cent incidence	23.07	61.29	6.45	22.58

of vitamin D to the incidence of parturient paresis. The effects of various prepartal amounts of vitamin D_2, fed at a daily rate of 30 million U.S.P. units, on the incidence of parturient paresis in animals with previous histories, are presented (Table 2). Our results show that in actual practice a reduction in the incidence of parturient paresis was greatest when experimental animals received a total prepartal amount of 60 million units. A total prepartal amount of 90 million units was next in effectiveness. When these data were grouped, it was found that the lowest incidence occurred when a total of 60 to 105 million units was fed prepartum.

As would be expected, the per cent incidence found for treatment animals without previous histories was considerably less than for treatment animals with previous histories. The results obtained on the effectiveness of various total prepartal amounts of vitamin D_2 fed show that a prepartal range of 15 to 45 million units afforded little protection, the incidence being significantly ($P > 0.01$) higher than the average for all groups. Further analysis of the data reveal that the 60–75 million unit range afforded the greatest protection, the incidence being significantly ($P > 0.01$) lower than the average for all groups. It seems apparent, in view of the data presented, that the total prepartal amount providing the greatest protection for animals with and without previous histories is within a range of 60–105 million units.

Animals that completed the prescribed treatment period one or more days prior to parturition were reassigned to the noncontinuous group (as shown in

TABLE 2

Effect of prepartal feeding of various total amounts of vitamin D_2 on the incidence of parturient paresis in dairy cows having previous histories

Million units of vitamin D_2	Total cows with previous histories	No. cases of parturient paresis
15	6	1
30	16	4
45	5	3
60	28	4
75	6	2
90	31	5
105	3	1
120	10	2
135	2	1
150	11	3
165	....	....
180	4	1
195	1	1
210	7	2
Total	130	30

Total units of vitamin D_2 fed prepartum	Total cows with previous histories	No. cases of parturient paresis	% Incidence
15– 45	27	8	29.62
60– 75	34	6	17.64
90–105	34	6	17.64
120–210	35	10	28.57
Total	130	30	23.37

TABLE 3

Effect of prepartal feeding of various total amounts of vitamin D_2 on the incidence of parturient paresis in dairy cows without previous histories

Million units of vitamin D_2	Total cows without previous histories	No. cases of parturient paresis
15	3	....
30	22	3
45	7	2
60	47	....
75	6	....
90	33	2
105	3	....
120	15	2
135	1	....
150	12	1
165	....	....
180	2	....
195	....	....
210	4	....
Total	155	10

Total units of vitamin D_2 fed prepartum	Total cows without previous histories	No. cases of parturient paresis	% Incidence
15– 45	32	5	15.62[a]
60– 75	53	....	00.00[a]
90–105	36	2	5.55
120–210	34	3	8.82
Total	155	10	6.45

[a] Difference significant at the ($P > 0.01$) level.

Table 4). All animals received a minimum of 60 million U.S.P. units of vitamin D_2 prepartum. In a few instances, experimental animals received a maximum of 210 million units prepartum. The data presented in Table 4 were excluded from all other tables. Although experimental numbers were small, the data show that the highest incidence of parturient paresis occurred in animals with previous histories when a total of 150–210 million units was fed prepartum.

It has been established, beyond a reasonable doubt, that factors which raise the level of blood calcium inhibit the activity of the parathyroids *(3, 4)*. Hibbs, Pounden, and Krauss *(16)* postulated that from one to five million units of vitamin D fed daily beginning 2–4 wk. prepartum suppressed parathyroid ac-

TABLE 4

Noncontinuous feeding of vitamin D_2 as related to the incidence of parturient paresis

Total units of vitamin D_2 fed prepartum	Total animals with previous histories	No. cases of parturient paresis	Total animals without previous histories	No. cases of parturient paresis
60– 75	1	1	1	1
90–135	3	1	3	....
150–210	10	6	6	
Total	14	8	10	1
	% Incidence 57.14		% Incidence 10.00	

tivity by the prolonged prepartal elevation of blood calcium levels. The high incidence of parturient paresis in previous-history animals in the noncontinuous group possibly may be attributed to prolonged suppression in parathyroid activity. Undoubtedly, the rate at which individual glands recover following partial or complete suppression will vary in accordance with the extent of serum hypercalcemia produced, as influenced by: the duration and amount of vitamin D consumed, the previous activity of the gland, the dietary calcium: phosphorus ratio, the age of the animal, or the metabolic state of the osseous tissue.

It is significant to note that in all instances parturient paresis developed when a lapse of from one to three days occurred between the cessation of treatment and parturition. It should be emphasized that a lapse of more than three days was sufficient to elicit the apparent recovery of the natural mechanisms involved in maintaining calcium homeostasis. Our results show that previous-history animals receiving the highest levels of vitamin D_2 prepartum exhibited the highest incidence of parturient paresis. Precalving parturient paresis was a common occurrence in animals included in this group.

These results may be interpreted as indicating that the parathyroids of previous-history animals are inherently less active and, therefore, more easily suppressed by large amounts of vitamin D_2. From a theoretical view, it seems reasonable to assume that following partial or complete gland suppression the duration of time required for recovery in susceptible animals may be greater than for nonsusceptible animals. The high incidence of parturient paresis in this group also suggests that the preventive action of vitamin D_2 is of limited duration following the termination of treatment.

Table 5 contains the results of administering 30 million U.S.P. units of vitamin D_2 per day on blood serum calcium and phosphorus levels. The prepartal and postpartal changes in blood serum calcium and phosphorus were very erratic and, because of the limited data available, it is difficult to describe any definite trends. It is interesting to note in this connection the marked individual variation that exists in blood serum calcium and phosphorus levels between multiparous dairy cattle of comparable ages. It should be emphasized, however, that the blood serum calcium and phosphorus levels found within 12 hr. prepartum for Animal A-3, and the postpartum levels found for Animal J-5, are within the critical range for parturient paresis, as previously determined by Greig *(10)*. These observations may be interpreted as indicating that the critical range at which parturient paresis manifests itself varies between breeds as well as within breeds.

The results of this investigation indicate an increase in blood serum phosphorus preceding that of blood serum calcium, and are in agreement with the results presented by Conrad, Hansard, and Hibbs *(5)*. The return of blood serum calcium to normal or above-normal levels following the termination of treatment in four experimental animals bled 48 hr. postpartum suggests the return of parathyroid activity. There is evidence that on the third day following five days of feeding vitamin D_2 to primiparous lactating Jersey cows, at a daily rate of 30 million U.S.P. units, blood serum calcium increased to a level

TABLE 5

Effect of vitamin D_2 on the blood serum calcium and phosphorus levels of normally parturient dairy cattle

Cow No.	Prefeeding		Approximately 36 hr. post-feeding		Within 12 hr. prepartum		Within 12 hr. postpartum		Approximately 24 hr. postpartum		Approximately 48 hr. postpartum	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
J–1	10.83	5.84	.....	.....	9.32	4.02	10.73	6.99	.....	.....	11.41	7.58
H–2*	9.68	6.12	9.22	7.24	.....	.....	9.87	6.01	9.70	5.47	.....	.....
A–3	9.09	5.03	9.02	6.57	4.65	3.29	6.72	5.27	8.80	7.58	.....	.....
H–4*	9.57	6.43	10.06	6.34	9.70	7.53	9.65	8.83	7.88	8.26	.....	.....
J–5*	7.30	5.60	7.32	6.75	7.84	7.82	5.75	5.17	5.26	3.26	.....	.....
J–6	7.43	6.80	6.52	6.93	5.65	4.25	8.34	6.59	6.68	9.34	.....	.....
J–7	9.76	5.99	10.21	6.72	8.91	6.52	7.91	6.25	7.43	5.91	8.96	5.44
J–8	9.20	7.33	9.17	7.47	.....	.....	6.80	9.20	9.18	9.01	10.18	10.02
A 9	6.13	6.04	8.27	8.40	8.49	8.47	5.91	6.75	7.38	6.37	8.20	5.72
J–10	8.46	6.10	9.09	6.28	9.88	8.28	8.17	7.89	.....	.....	.....	.....
Average	9.10	6.14	8.78	6.86	7.67	5.57	8.22	6.78	7.84	6.97	10.18	7.66

* Indicates a previous history of parturient paresis.

slightly above normal *(6)*. Blood serum phosphorus levels declined during this same period. This evidence may be interpreted as indicating that the mobilization of calcium was occurring through the return of parathyroid activity rather than by the residual action of previously administered vitamin D_2.

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METHOXYL AS AN INDICATOR OF THE NUTRITIVE VALUE OF FORAGE [1]

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SUMMARY

A study was made of the relationships between the digestibility and methoxyl content of forages and between forage digestibility and the fecal methoxyl content. The three forages studied were of different botanical composition, were both grazed and hand-fed, and each was harvested at several stages of growth. The coefficients of digestibility of the clipped forage were determined in the conventional digestion trial, whereas those of the grazed forage were estimated by the chromogen technique.

The digestibility of the dry matter and the methoxyl content of the hand-fed forage were highly significantly correlated ($r = -0.83$). The methoxyl content of forage appears to be useful as a relative index of forage digestibility for selecting among similar forages of widely different digestibility.

The regression of dry matter digestibility on the concentration of methoxyl in feces was not statistically different for the three forage mixtures or for grazed and hand-fed forage. The pooled regression of percentage dry matter digestibility *(Y)* on percentage fecal methoxyl *(X)*, representing all forages (*i.e.*, grazed and hand-fed), is: $Y = 94.18 - 9.586X$, and the correlation coefficient obtained for 107 observations is $-.742$.

In the recent past, notable achievements have been made in development of research tools to aid plant and animal investigators in evaluating, under grazing, the yield and nutritive value of pasture swards. Chief among the useful techniques are the plant chromogens method for digestibility and the chromic oxide method for fecal outgo *(2, 8, 10, 11, 12, 18, 19, 20, 24)*. Lignin as a component of plant materials has been studied largely because of its role in lowering the nutritive value of plant materials *(1, 5, 6, 7)*. However, methods for estimating lignin *(4)* are laborious and results often are more variable than desirable for a method to be used in definitive studies.

Methoxyl groups are contained in lignin in a very firm attachment *(13, 17, 23, 26)* and the methoxyl content of plants increases as the plants mature *(6, 15, 16, 27)*. As plants mature, not only does the concentration of lignin increase, but also there is an increase in methoxyl radicals on the lignin molecule *(14, 15, 16)*. It would appear, therefore, that the methoxyl group might be a useful index of forage quality. Richards and Reid *(21, 22)* and Kane *et al. (9)* showed that forage methoxyl was digested but that the digestion of the material decreased as the plant became more mature. Richards and Reid *(21, 22)* found

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a highly significant negative correlation to exist between the methoxyl content of feces of grazing animals and the digestible dry matter content of the forage. This study suggested the possibility of predicting digestibility of forage dry matter from a knowledge of the fecal methoxyl content in feces of free-grazing cattle.

The present study was carried out to determine the relationship of fecal methoxyl to dry matter digestibility, and between forage digestibility and forage methoxyl, for several kinds of forage crops grazed and harvested and fed at several stages of maturity.

EXPERIMENTAL PROCEDURE

A complete description of the forages studied, and the methods used for feeding animals and collecting feces, have been described by Reid *et al. (19)*. Two methods of feeding were practiced. Clipped, whole forages of different botanical composition and of several stages of maturity were hand-fed to steers maintained in metabolism stalls. At the same time similar forages, grown on areas adjacent to the forage used for clipping, were grazed. During the 1949 season, a timothy-mixed grass forage was studied at periods when the timothy was in the vegetative, boot-to-early head, and full-bloom stages. In 1950, three different kinds of forage were studied; these will be referred to as Forages 1, 2, and 3. Forage 1 was predominantly Kentucky bluegrass and timothy. This forage was studied simultaneously by grazing and hand-feeding during four stages of maturity (Trials 1–4). A fifth grazing trial was conducted on aftermath. Forage 2 was a mixed sward consisting of timothy, quack grass, alfalfa, and clover. Forage Mixture 3 consisted of quack grass, timothy, alfalfa, clover, and Kentucky bluegrass. Forage Mixtures 2 and 3 were clipped, hand-fed, and grazed during three stages of uninterrupted growth and during two aftermath stages (Trials 1–5). For Forage 1 in the fifth grazing period, inadequate yield necessitated the harvesting of forage from another area for the hand-fed animals. Forage 3 was used and the trial is designated 5A. Dairy-type growing steers were employed as test animals and they ranged in weight from 336 to 438 lb. For each forage test, there were three "outside" animals and three "inside" animals. The terms outside and inside will be employed hereafter to refer to grazing and hand-fed animals, respectively.

The methoxyl content of the feed and feces was estimated by a modification of the Zeisel method adapted by the U. S. Forest Products Laboratory *(3)*. The second absorption tube was not used and the regular absorption tube assembly was replaced by a larger tube. Fifteen milliliters of $AgNO_3$ were used in the single tube. A wooden frame was designed and built to accommodate eight complete digestion assemblies. Materials for analysis were weighed in tared gelatin capsules.

RESULTS AND DISCUSSION

In all hand-feeding trials the mean recovery of forage methoxyl in the feces was 63.52%, with a standard deviation of 4.16%. This is in agreement with the report of Kane *et al. (9)*, who found the methoxyl of orchardgrass to be

digested to the extent of 50% by cattle. In the present study, digestibility declined as the plants matured. In Table 1 it may be noted that in the first digestion trial for all forages, methoxyl recovery averaged only 50.80%; whereas, at the time the third digestion trials were conducted, methoxyl recovery amounted to 75.14%. In the meantime, dry matter digestibility had declined from 72.35 to 54.93%.

The regressions of the mean dry matter digestibility on forage methoxyl for each forage and for each stage of maturity were calculated. These data are summarized (Table 2). The correlation coefficients for Forages 1, 2, and 3 between dry matter digestibility and methoxyl content of the forages are −.883, −.925, and −.858, respectively. An analysis of errors of estimate from average regression within forages revealed no significant differences among the three regressions. Because the relationship between dry matter digestibility and forage methoxyl was not statistically different for the three forages, the data for all three forages were pooled and the relationship between dry matter digesti-

TABLE 1

Methoxyl content and digestibility of dry matter of forages and rate of recovery of ingested methoxyl from feces

No. Forage	Trial No.	Methoxyl content	Methoxyl[a] recovery	Dry matter digestibility
			(%)	
1 (1949)	1	1.58	44.80	72.9
	2	1.75	56.50	66.3
	3	2.10	68.60	58.0
1 (1950)	1	1.49	58.72	72.7
	2	1.80	65.43	66.3
	3	1.87	95.34	54.4
	4	2.24	80.82	54.8
2 (1950)	1	1.74	43.06	72.1
	2	2.58	55.64	59.3
	3	3.05	63.79	53.5
	4	1.93	57.32	63.6
	5	2.09	62.88	61.8
3 (1950)	1	1.50	56.60	71.6
	2	1.73	85.02	63.2
	3	2.60	72.83	53.8
	4	2.09	59.42	60.4
	5	2.06	67.29	60.1
	5A	2.60	49.25	60.2
			Mean 63.52 ± 4.16	

[a] Values given are the means of data obtained with three steers.

TABLE 2

Covariance analysis of the relationship of dry matter digestibility (%), Y, to methoxyl content (%) of forage, X (hand-fed trials)

Forage No.	Degrees of freedom	S_x2	S_{xy}	S_y2	r	b
1	6	0.4279	−11.2371	378.7876	−.883	−26.261
2	4	1.1331	−13.3518	184.2372	−.925	−11.783
3	5	0.9985	−11.1623	169.4904	−.858	−11.179
Sum	15	2.5595	−35.7512	732.5152	−.826	−13.968[a]

[a] $S_b = 2.755$.

bility and forage methoxyl was expressed in one equation. This relationship is shown graphically in Figure 1. A line expressing this relationship is determined by the equation, $Y = 91.01 - 13.968X$, where Y is the dry matter digestibility (%) and X is the methoxyl content (%) of the forage dry matter. The standard deviation for the regression coefficient is 2.755. The correlation coefficient for the relationship was −.826 (99% confidence limits are −.430 to −.956). These data show conclusively that for these forages methoxyl content was associated closely with feeding value.

The next relationship to be considered was that between fecal methoxyl and the digestibility of the forage dry matter. To evaluate this relationship, both the hand-fed and grazing steer data were employed. Regression lines calculated

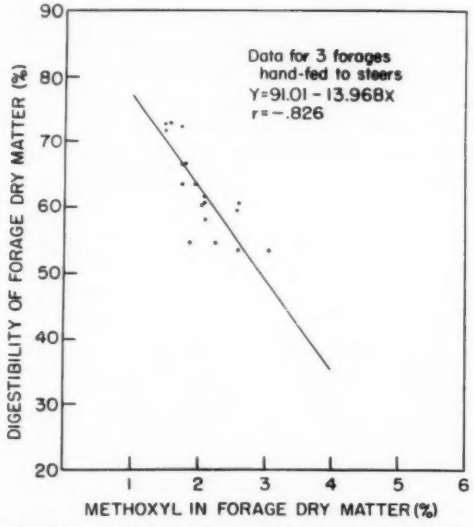

FIG. 1. Relationship of methoxyl level of forage to forage digestibility.

for each forage hand-fed to steers were found not to be statistically different, and the pooled regression for the three forages (hand-fed) adequately expressed the relationship of forage digestibility and fecal methoxyl. The regression equation, calculated from the sums of the individual regressions of dry matter digestibility (%), Y, on fecal methoxyl (%), X, is $Y = 96.40 - 9.893X$.

The individual regressions of dry matter digestibility on fecal methoxyl for steers that grazed the three kinds of forage are summarized (Table 3). The correlation coefficients for the same relationship also are given. These correlation coefficients are −.825, −.624, and −.771, respectively, for Forages 1, 2, and 3. The probability of a greater "r" for any single forage by random sampling is less than one per 100 samplings. Covariance analysis showed no real difference to exist among the individual regressions. Accordingly, the average regression with 47 degrees of freedom is a better expression of the relationship than any

TABLE 3

Covariance analysis of the relationship of dry matter digestibility (%), Y, to fecal methoxyl (%), X (free grazing steers)

Forage No.	Degrees of freedom	S_x2	S_{xy}	S_y2	r	b
1	22	4.0060	−52.0743	1097.0238	−.825	−12.999
2	14	3.2286	−23.8712	453.6248	−.624	− 7.394
3	14	3.6795	−22.5745	232.8702	−.771	− 6.135
Sum	50	10.9141	−98.5200	1783.5188	−.706	− 9.027[a]

[a] $S_b = 1.3203$.

one individual regression. This average regression was calculated from the sums of the individual regressions (Table 3). The regression equation is, $Y = 91.24 - 9.027X$, where Y is the dry matter digestibility (%) and X is the fecal methoxyl (%). The standard deviation of "b" is 1.3203.

Finally, it was of interest to determine if the regressions of dry matter digestibility on fecal methoxyl were different for the inside and outside trials. On applying an appropriate statistical test *(25)*, differences were found not to be significant. Consequently, the relationship between dry matter digestibility and fecal methoxyl was the same, regardless of whether inside or outside trials were considered.

Since, in this study, the individual regressions for the three forages were not different, and since the regressions for the data obtained from inside and outside trials are the same, all observations for all forages may be pooled into a single regression equation (Figure 2). The regression of dry matter digestibility (%), Y, on fecal methoxyl (%), X, was found to be $Y = 94.17 - 9.586\,X$. The standard deviation of "b" is 0.8780. The correlation coefficient for 107 observations between dry matter digestibility and fecal methoxyl was −.742. The 99% confidence limits for the correlation coefficient are −.595 to −.840.

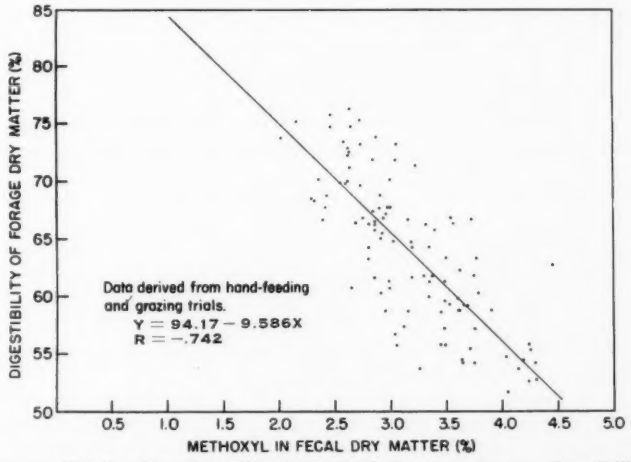

FIG. 2. Relationship of methoxyl level of feces to forage digestibility.

These data confirm the report of Richards and Reid that the digestibility of forage by cattle is closely correlated in a negative manner with fecal methoxyl. The correlation coefficient found for this relationship in this investigation is not as large as that reported by Richards and Reid *(22)*, and the scatter of observations around the regression line is more variable. However, the data obtained in the present investigation represent a study of several forages of widely different botanical composition and of several stages of maturity. The data presented in the earlier report *(22)* were obtained from a study of one pasture herbage during three stages of maturity.

Although fecal methoxyl is correlated in a highly significant manner with forage digestibility it does not appear, from the data of this study, that fecal methoxyl could be used with confidence to predict forage digestibility. The reasons for this conclusion are *(a)* the range within which digestibility could be predicted is too wide for the precision required in research work; *(b)* the range within which methoxyl values may vary for the common pasture species is too limited, and *(c)* the repeatability of the methoxyl determinations may affect the results. The second factor is of great importance, because a slight error in estimating methoxyl leads to a great error in estimations of digestibility. In this study, a decrease in forage digestibility of 19.3 percentage units was associated with an increase in fecal methoxyl of only 1.74 percentage units. In the usual volumetric procedure for determining methoxyl, the end-point of the titration is not sharp and may be missed rather often, even by experienced personnel. Furthermore, the method does not discern between the methoxyl groups of lignin and those of other substances, or between ethoxyl and methoxyl groups. The methoxyl group of free methionine is measured quantitatively as methoxyl. In addition to these limitations, it must be recognized that the chromogen technique *(19)* has been proven to be an accurate and expedient means of estimating forage digestibility by grazing animals. The availability of a more precise alternate method, therefore, precludes wide use of fecal methoxyl as an indicator of forage digestibility.

Nevertheless, because of the simplicity with which methoxyl may be estimated, and because of its high correlation with forage digestibility, methoxyl in plants and feces should become a useful research tool for plant breeders, animal nutritionists, and others, for screening materials for more definitive studies.

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EXCRETION OF CHROMIUM OXIDE BY DAIRY COWS

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SUMMARY

An experiment was conducted to study the effects of feeding schedule and various ratios of forage to concentrates upon the excretion pattern of Cr_2O_3. The indicator was administered once daily to Holstein cows consuming rations containing from 35 to 100% roughage. Fecal samples were taken every 2 hr. during the first two periods and every 3 hr. during the last two periods. Chromium oxide per gram of fecal dry matter was determined on all samples.

A symmetrical excretion pattern was observed when average values were plotted, and an equation was fitted to the excretion curve. There was considerable variation in relative Cr_2O_3 concentration at any sampling time, indicating the importance of obtaining a large number of samples; however, neither the feeding schedule nor the proportion of roughage to concentrate had an influence upon the general character of the curve.

It was concluded that time of Cr_2O_3 administration was of primary importance in respect to time-concentration relationships of fecal Cr_2O_3, and that so-called physiological, diurnal effects were of little importance. The data suggest that a 12-hr. sampling procedure would be more accurate than unequal fecal sampling times.

The literature concerned with indigestible indicators and fecal indices has been reviewed *(25, 39)*, as have the factors affecting the rate of excretion of several different markers when administered to ruminants *(33)*. The excretion curve for ruminants described in the literature reviewed by Mäkela *(33)* has been shown to be characteristic of those species and it is presumed to be a result of the "buffering action" of the rumen *(23, 30, 36)*.

Variations with time in fecal concentration of Cr_2O_3 during periodic Cr_2O_3 administration have been described by a number of workers *(5, 15, 20, 21, 26, 28, 31, 34, 37, 38, 42)*. Differences in concentration were greatest when Cr_2O_3 was given once daily, and were nearly eliminated when six doses were given per day. It has been observed that the density of the indicator is of no importance as long as particle size is small *(30, 38)*.

Since it takes about four days for a dose of Cr_2O_3 to pass through the ruminant digestive tract *(19, 31, 38)*, a preliminary Cr_2O_3 administration period of from seven to ten days has been considered sufficient *(16, 21, 26, 34, 42)*. The length of the collection period has not been as well defined, although Smith and Reid *(42)* have obtained good results when sampling twice a day for seven days.

Only a few experiment stations *(1, 2, 17, 24)* have been using the Cr_2O_3 indicator method routinely, and these have employed Edin's method instead of the grab-sampling procedure popular in this country. As a result, it is difficult to compare the "routine accuracy" of the indicator and total collection

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methods. The indicator method is usually assumed to be less accurate than total fecal collection. Errors inherent in the indicator method may be associated with the measurement of Cr_2O_3 intake *(9, 13)* or excretion *(34)*, irregular rate of passage *(8, 11, 30)*, or retention of the indicator *(4)*. Further errors may be associated with the analytical procedures employed. The method originally described by Edin *et al. (16)* was slow and laborious, so they proposed employing perchloric acid as an oxidizing reagent. The latter method was rapid but was not considered as accurate as the former. Other workers have described modifications of the perchloric acid method *(6, 29)* and have reported upon various sources of error *(3, 22, 40)*. Fecal Cr_2O_3 has also been determined by use of the alkaline fusion method, which has been used and modified by a number of workers *(4, 7, 14, 18, 35, 41)*. The use of potassium bromate as an oxidizing agent has been described by Coup and Lancaster *(12)* and the method has been simplified by Christian and Coup *(10)*. The potassium bromate method is more rapid than the alkaline fusion method and is apparently not as subject to error as is the perchloric method. Kane *et al. (27)* recently have used radioactive chromium ($Cr_2{}^{51}O_3$) to determine digestibilty.

The variable results reported in fecal concentration and recovery of Cr_2O_3 suggested the desirability of defining the factors which cause the variation. This study was planned to measure the influence of feeding schedule, time of sampling the excreta, and wide differences in type of rations upon the excretion pattern of Cr_2O_3.

EXPERIMENTAL PROCEDURE

The experimental animals were mature, purebred Holstein cows. Rations fed contained 100, 75, 50, and 35% roughage on an air-dry weight basis. The roughage component of the ration consisted of alfalfa hay in half of the trials and alfalfa hay-corn silage on a 1 : 3 ratio, as-fed basis in the others. The animals were given all the feed they would eat, with the above-mentioned ratios being maintained.

Twenty grams of Cr_2O_3 (Baker C.P.) were administered in gelatin capsules each day at 2 P.M., for at least ten days before and during each collection period. Fecal grab samples, weighing from 200 to 400 g., were taken rectally every 2 hr. during the first two periods and every 3 hr. during the last two periods. Each period was two days in length. An aliquot from the daily total fecal collections was obtained during part of the trials. The fecal samples were dried at approximately 60° C. for 48 hr. After being allowed to come to equilibrium with atmospheric moisture, the samples were ground in a Wiley mill and placed in screw-capped sample jars from which samples were taken for chemical analyses. Analytical dry matter was determined by drying for 24 hr. at 100° C. The Cr_2O_3 content per gram of dry feces was determined by the method of Christian and Coup *(10)*, as employed by the USDA Beltsville laboratory.

RESULTS AND DISCUSSION

For comparative purposes, the milligrams of Cr_2O_3 per gram of fecal dry matter were converted to percentage of the daily mean concentration. When

all the values for each sampling time were averaged and plotted (Figure 1) a relatively smooth and symmetrical curve resulted. An equation which fitted the average values well was $E(Y) = 100.44 + 12.84 \sin 15° (X - 8 \text{ am})$, where X is hours after midnight and Y is the percentage of the daily mean fecal Cr_2O_3 concentration. If individual observations deviated but little from the curve described by this equation, it would be possible to sample once a day and to correct the observed values to the daily mean Cr_2O_3 concentration. Unfor-

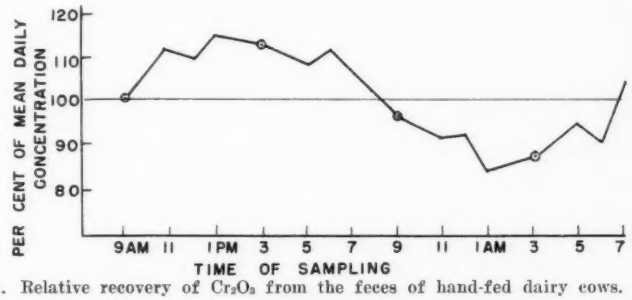

FIG. 1. Relative recovery of Cr_2O_3 from the feces of hand-fed dairy cows. Each point represents the average of 16 samples and each circled point, 32 samples.

tunately, the individual observations at any one sampling time were extremely variable. The curve described by the above equation is shown (Figure 2), as is the variability in fecal Cr_2O_3 concentration among individual grab samples. It is somewhat surprising that when these values were averaged for each sample time by periods, treatments, or animals, the resulting curves were all similar

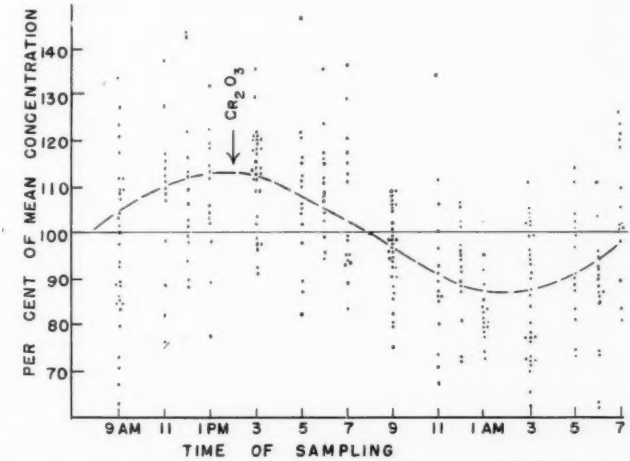

FIG. 2. Regression curve of Cr_2O_3 recovery on time. Each dot represents a single sample. $E(Y) = 100.44 + 12.84 \sin 15° (X-8 \text{ am})$, where X is hours after 12 midnight and Y is the percentage of the daily mean fecal Cr_2O_3 concentration.

in character. Nevertheless, such variation (Figure 2) emphasizes the importance of obtaining a large number of fecal grab samples.

The excretion curve observed in this experiment was compared (Figure 3) with those reported by Kane *et al. (28)* and Hardison and Reid *(21)*. Times of Cr_2O_3 administration were used as common points when aligning the curves. The similarity of the curves is striking and suggests that time of Cr_2O_3 administration is of primary importance in respect to time-concentration relationships of fecal Cr_2O_3. In contrast to the views of some other workers *(5, 15, 28)*, it appears that the "inherent periodicity in the chromic oxide excretion pattern" is easily altered by varying the time of Cr_2O_3 administration.

The percentage recovery of Cr_2O_3 for two periods was 99.6%, with a standard error of the mean of 2.7%. The variability in per cent recovery among animal-days was similar to that reported by Pigden and Brisson *(37)*, and ranged from 85 to 124%. Part of the variability is probably associated with the recognized variation in the total amount of feces voided from day to day. In general, the data indicate that a consistent excretion curve and approximately 100% recovery of Cr_2O_3 can be obtained when rations varying widely in proportion of roughage to concentrate are consumed by hand-fed dairy cows, and that time of feeding has little or no effect.

The data suggest that seven-day collections are sufficient for estimating time-concentration trends for fecal Cr_2O_3, but recovery values suggest that a longer sampling period might be desirable. Sampling times should be selected so that the most accurate estimate of digestibility may be obtained; that is, the mean daily fecal Cr_2O_3 concentration must be determined. By observing the curve in Figure 2, it may be seen that combining samples obtained at 12-hr.

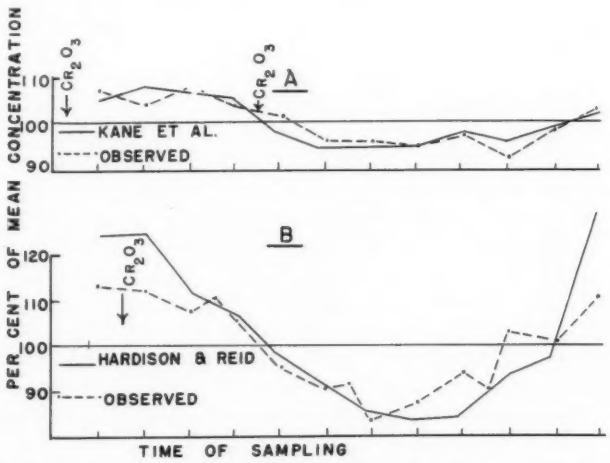

FIG. 3. A. Comparison of the observed excretion curve with that of Kane *et al. (28)*. Our data were plotted as if the Cr_2O_3 had been administered two times daily—see Lambourne *(32)*. B. Comparison of the observed excretion curve with that of Hardison and Reid *(21)*.

intervals will give the desired values, regardless of when the samples are taken. In addition, such a procedure has the advantage of not being invalidated if the alignment of the curve changes along the time axis, or if the amplitude should vary with treatments and animals, as long as the symmetry of the curve is maintained.

If the above assumptions are correct, a seven-day collection period with once-a-day Cr_2O_3 administration, a 12-hourly grab-sampling schedule, and four animals should result in digestibility values within $\pm 5\%$ of those obtained by the total collection procedure. As Hardison *et al. (20)* have suggested, more accurate results may be obtained if Cr_2O_3 is administered twice a day and animal numbers per treatment are increased.

To test the above assumptions, a comparison of the dry matter digestibility by dairy cows as measured by complete fecal collections and by grab samples was made. The results are shown (Table 1). Fecal grabs were taken at 3 A.M. and 3 P.M. Chromic oxide was administered at 2 P.M. The data show no advantage for a 14-day grab-sampling period over a seven-day period. All of the results using Cr_2O_3 were lower than the results from complete collections, but are within the predicted error range *(32)*. Theoretically, a 12-hr. sampling procedure is desirable. Practically, the 10- and 14-hr. intervals for sampling *(42)* are preferable and, under the conditions of this experiment, would bias the results only by 1 or 2%.

TABLE 1

Digestible dry matter as determined by total collection and the indicator method

Cow No.	Total collection method	Indicator method	
		7-day	14-day
177	66.1	65.7	63.8
196	69.3	66.2	68.1
166	70.1	67.5	68.3
279	69.3	64.2	62.8
	Average 68.7	65.9	65.8

The indicator method at its best is an estimate of values which at the present time can be obtained only by the total collection procedure. Thus, the use of the indicator method depends upon the degree of accuracy desired. Since the indicator method should give results at least as accurate as, and perhaps more accurate than, average or calculated digestibility values, its use would appear preferable where conditions prohibit the total collection procedure and digestibility values are desired.

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COMPARISON OF ALFALFA FED AS SOILAGE OR SILAGE AND THE ONLY FORAGE FOR LACTATING DAIRY COWS [1]

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SUMMARY

Twenty Holstein and Brown Swiss cows were fed ad libitum silage previously made from first-crop alfalfa, or soilage from second- and third-crop alfalfa and grain, at the rate of 1 lb. for each 4 lb. of milk based on milk yield during a 2-wk. pre-experimental period.

The cows fed soilage consumed more total dry matter and maintained a higher level of milk production than did those fed silage. However, the cows fed silage were slightly more efficient in converting nutrients to milk. During the experimental period, milk from cows fed silage increased slightly in average butterfat content, whereas that from cows fed soilage decreased somewhat. Total solids content of milk from both groups increased rather uniformly. At the completion of the post-experimental period, both groups were producing at almost the same level, with essentially the same relative difference in butterfat and total solids percentages as they had during the pre-experimental period.

Soilage has long been one of the methods used for feeding forages. Kildee *et al. (8)* reviewed the early literature on feeding soilage, rather than grazing, as a method of pasture management. It was concluded that, although this system fits many conditions, the labor requirements were too high. With the modern machinery available on most dairy farms, the practice of soiling, or green-chop feeding, is now being widely used. Although there are many reports in the early literature on soilage for dairy cows, Graves *et al. (6)* made the first recorded comparison wherein pasture crops were grazed, fed as soilage, as silage, and as hay. Donker *(4)* reported that there was little difference in the milk production from cows fed first-crop alfalfa-brome and oats put up as silage, and those fed second-crop alfalfa-brome, oats, and Sudan grass as soilage.

In a recent report, Huffman *et al. (7)* concluded that immature alfalfa fed as soilage was approximately equal (on a dry matter basis), in grain-equivalent for milk production to good-quality alfalfa silage made from an adjoining strip, when they replaced all or part of a hay and grain ration.

Although a surplus of pasture is usual during the early part of the summer, one of the problems on many farms is providing sufficient pasture during the hot, dry months of July and August. A possible solution is to ensile at least part of the first hay crop to supplement pasture later in the summer.

The purpose of this experiment was to compare the effect on milk production of feeding first-crop alfalfa put up as silage with second- and third-crop alfalfa fed as soilage.

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EXPERIMENTAL PROCEDURE

A relatively pure stand of first-crop, mid-bloom alfalfa was ensiled in a portable bunker silo, on June 22, 1957. Sodium metabisulfite[2] (10 lb/ton) was added by hand-broadcasting and was mixed into the forage with silage forks just before packing with a tractor. Three grab samples were taken from each load of the chopped forage as it was put in the silo. Samples from the first third of the total number of loads were pooled and a representative sample taken for proximate analysis *(1)*. This was repeated for the loads representing the middle and the top segments of the silo. The silage was of good quality based on color, odor, and chemical analyses. The soilage was pre-bloom to early-bloom second- and third-crop alfalfa, chopped once daily in the early morning, and fed in a hay bunk with a heavy screen bottom to permit air circulation and reduce heating.

At the time of feeding each morning, at least 20 grab samples were taken from the silage and from the soilage. Representative samples of each type of forage were placed in separate plastic bags. This procedure was repeated daily until pooled weekly samples were obtained. Proximate analyses *(1)* were made on the pooled samples of soilage and of silage each week of the experiment. Dry matter analyses were made on soilage and silage as fed and on both materials that remained in the feed bunks the next morning. From these data the mean dry matter consumption per cow was determined.

Twenty Holstein and Brown Swiss cows were used in this study. They were paired by breed and as closely as possible according to milk production, stage of lactation, and body weight. One animal from each pair was randomly assigned to the soilage, and the other to the silage, group. All cows grazed brome pasture during a 2-wk. pre-experimental period, when base milk production was determined. The experiment was conducted over an 8-wk. period (July 8 to September 1, 1957). The cows then were returned to a brome pasture and milk production observations were made for a 2-wk. post-experimental period. A concentrate mixture (ground shelled corn, 500; crushed oats, 300; wheat bran, 400; soybean oil meal, 200; salt, 14; steamed bone meal, 14; calcium carbonate, 14) was fed at the rate of 1 lb. for each 4 lb. of milk produced during the base period and at a constant level thereafter.

Daily milk weights were recorded and milk samples were taken weekly. The butterfat was determined by the Babcock test and total solids by the AOAC method *(1)*.

Body weights were determined by averaging weights on two consecutive days at the start, at the middle, and at the end of the experimental period. Mean changes in body weight at 4 and 8 wk., respectively, were: soilage groups, −1 and −72, and silage groups, +30 and −52. Daily TDN intake above maintenance requirements was adjusted to account for gain or loss of body weight *(9)*.

TDN values were estimated by employing digestion coefficients for soilage

[2] Provided by Monsanto Chemical Co., Boston, Mass., and General Chemical Co., New York, N. Y.

(alfalfa, green, all analyses) *(10)*, for concentrates *(10)*, and for silage (first-crop, 8 lb. sodium metabisulfite) *(2)*.

RESULTS

The effect of source of forage on milk production (actual and FCM) and on milk composition is shown (Table 1). The level of milk production during the pre-experimental period was almost identical in the two groups of cows. However, a slightly higher butterfat test in the group assigned to soilage resulted in an average pre-experimental 4% FCM yield of 39.59 lb., as contrasted with 37.87 lb. for the group to be fed silage. Both groups produced at lower levels on the experimental rations and the mean daily differences in observed milk yield of 5.82 lb. and in FCM of 4.93 lb. in favor of the soilage group were significant ($P = .05$). When these differences were adjusted by covariance on performance during the preliminary period, the difference in actual yield was significant ($P = .05$), and the difference in 4% FCM was highly significant ($P = .01$).

The gains in efficiency from pairing based on milk production during the 2-wk. preliminary period were: 304, 127, 78, and 203% for actual pounds milk production, per cent total solids, per cent fat, and pounds 4% FCM, respectively. These gains in efficiency were relative to assigning the cows completely at random to the rations. Additional gains in efficiency from using covariance of response during the experimental period on the corresponding response during the preliminary period were: 2.5, 76.6, 31.5, and 10.1% for actual pounds milk production, per cent total solids, per cent fat, and pounds 4% FCM, respectively.

The trends in mean daily milk production are shown (Figure 1). Subsequent to the first week of the experimental period, differences in mean daily milk production between the two groups of cows were fairly constant.

During the post-experimental period, the differences observed in mean per cent total solids and per cent fat between the two groups were essentially the same as those observed during the pre-experimental period (Table 1). Although the mean daily milk production during the 2-wk. post-experimental period favored the soilage group, Figure 1 shows that milk production during the last week of this period was essentially the same for both groups of cows.

Although cows fed soilage consumed more total dry matter (Table 2) as forage and produced more milk (actual and 4%FCM), they were somewhat less efficient in milk production than those fed silage. The cows fed soilage produced an average of 1.83 lb. of 4% FCM per pound of TDN consumed (above maintenance); whereas, those fed silage produced an average of 2.12 lb. of 4% FCM for each pound of TDN intake (above maintenance).

The composition of soilage and silage fed in this experiment is shown on a weekly basis in Table 3. Values for silage were somewhat more consistent than those for soilage. The dry matter values for the latter varied from 15.61 to 26.55%, whereas, the former ranged from 22.92 to 25.50%. The mean calculated TDN was 13.09% for soilage and 13.57 for the silage. The soilage was slightly

TABLE 1

Effect of type of forage consumed on mean milk production and composition

	Pounds of milk				Total solids				Fat				F.C.M.			
	Soil-age	Si-lage	Differ-ence	SED[a]	Soil-age	Si-lage	Differ-ence	SED	Soil-age	Si-lage	Differ-ence	SED	Soil-age	Si-lage	Differ-ence	SED
	(lb/cow/day)				*(%)*				*(%)*				*(lb/cow/day)*			
Pre-experimental	45.73	45.58	0.15	0.81	11.43	10.86	0.57	0.36	3.14	2.84	0.30	0.20	39.59	37.87	1.72	1.95
Experimental																
Observed	43.32	36.50	5.82[c]	1.64	11.62	11.13	0.49	0.22	3.09	3.06	0.03	0.13	36.20	31.27	4.93[c]	1.41
Adjusted[b]	42.27	36.56	5.71[c]	1.62	11.48	11.26	0.22	0.17	3.02	3.12	−0.10	0.11	35.89	31.58	4.31[d]	1.36
Post-experimental	34.34	33.32	1.02	1.36	11.84	11.21	0.63	0.28	3.52	3.16	0.36	0.20	31.53	28.92	2.61	1.59

[a] SED = Standard error of difference.
[b] Adjusted by covariance on performance during preliminary period.
[c] Significant at P = .05.
[d] Significant at P = .01.

TABLE 2

Mean daily forage consumption[a]

Week	Soilage		Silage	
	As fed	DM	As fed	DM
	(lb.)			
1	125	26.2	82	19.8
2	96	22.9	82	20.8
3	96	25.4	88	21.7
4	100	23.8	85	20.8
5	130	27.8	98	23.8
6	134	27.0	97	23.8
7	148	27.8	99	23.8
8	156	24.4	101	22.9
Mean	123	25.7	91.5	22.2

[a] Total consumption/1,000 lb. body weight.

higher than the silage in per cent crude protein and NFE, but lower in ash, ether extract, crude fiber, and calculated TDN.

The average daily forage consumption by weeks on a 1,000-lb. body weight

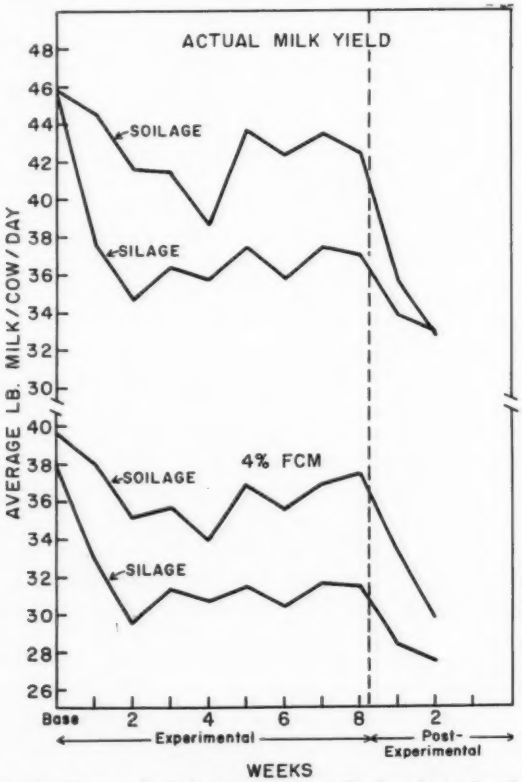

FIG. 1. Effect of soilage and of silage on milk production (actual and 4% FCM).

TABLE 3

Proximate analysis of alfalfa soilage and silage

Week	Soilage							Silage						
	DM	Ash	CP	EE	CF	NFE	Calculated TDN	DM	Ash	CP	EE	CF	NFE	Calculated TDN
	(%)	*(% DM basis)*					*(%)*	*(%)*	*(%, DM basis)*					*(%)*
1	20.99	8.94	21.09	2.20	25.97	41.80	12.86	25.50	11.88	17.25	3.39	28.80	38.68	14.01
2	23.90	8.29	19.41	1.73	26.18	44.39	14.72	25.48	11.72	18.64	2.98	30.68	35.93	13.92
3	26.55	7.26	16.75	1.77	30.11	44.11	16.27	24.52	11.43	18.66	3.38	30.30	36.23	13.52
4	23.77	8.06	19.25	1.57	28.70	42.99	14.59	25.10	11.48	16.47	3.49	31.69	36.87	13.81
5	21.32	9.61	21.34	2.11	23.95	42.99	13.05	24.41	11.05	17.50	3.72	31.14	36.59	13.55
6	20.20	10.22	19.53	2.69	26.13	41.43	12.15	24.56	11.03	18.68	3.32	32.61	34.36	13.56
7	18.78	9.89	23.44	2.04	22.79	41.84	11.53	24.46	11.26	15.81	3.20	35.46	34.27	13.33
8	15.61	10.00	23.60	2.22	24.47	39.71	9.51	22.92	11.49	16.47	4.01	34.13	33.90	12.65
Average	21.39	9.03	20.55	2.04	26.04	42.41	13.09	24.62	11.44	17.44	3.44	31.85	35.85	13.57

basis is shown (Table 2). The soilage group consumed a daily average of 123 lb., compared with 91.5 lb. for the silage group. Since the silage was 24.62% dry matter compared with 21.39% for the soilage, the cows fed soilage consumed 25.7 lb. of dry matter per 1,000 lb. body weight daily, as contrasted to only 22.2 lb. by cows in the other group.

DISCUSSION

When two groups of dairy cows of equal milk-producing ability were fed a constant amount of grain and either alfalfa soilage or alfalfa silage ad libitum, the cows fed soilage maintained a higher level of milk production, although after the first week of the experiment the weekly variation in milk production between the two groups was fairly uniform (as shown in Figure 1). Similar differences have been reported in previous work at this station *(5)*, where cows fed soilage produced 45.6 lb., and those fed silage 41.3 lb., milk per animal per day during a 4-wk. trial. The cows fed soilage consumed more total forage and more total dry matter than the cows fed silage. However, consumption levels of soilage were much more variable than silage, largely because of the wide variation in per cent dry matter. Apparently, the green forage was more palatable as the cows fed soilage consumed more total nutrients than those fed silage and produced more 4% FCM. This is in contrast to the work by Donker *(4)*, who reported that there was little difference in dry matter consumption of either soilage or silage by cows under Minnesota conditions, although the cows fed silage consumed more hay, resulting in a higher total dry matter intake. Essentially no difference was found in milk production between the cows on the two different rations, but there was some variation in the kind of forages fed as silage or soilage.

It was observed that the efficiency of production was somewhat greater in cows fed silage than in those consuming soilage. Although an explanation of the apparent increase in efficiency is not immediately available, the possibility of greater estrogenic activity in the silage may be considered. Pieterse and Andrews *(11)* report higher estrogenic activity in first-crop alfalfa than in later cuttings. Although the alfalfa put up as silage in this experiment was more mature than subsequent cuttings fed as soilage, the estrogenic activity of first-crop alfalfa cut at mid-bloom probably is greater than that of early-bloom second-cutting alfalfa *(11)*. Apparently, the ensiling process has no effect on estrogenic activity *(12)*. Although the effect of estrogens on milk production and utilization of nutrients has not been established adequately, Browning *et al. (3)* found that low-level feeding of diethylstilbestrol to identical twin cows resulted in a slight increase in milk production and efficiency of production.

The calculated TDN value for silage was higher and less variable than that for the soilage. There also was less variability in the percentage composition of the various nutrient constituents in the silage than in the soilage. Even though there was a gradual increase in nutrient intake in silage, and a more variable intake in soilage, the general pattern of mean daily milk production between the two groups was quite similar. This suggests that, once the milk production

values of the two forages have been established, weekly variations in production probably were due to factors other than source of nutrients.

The sudden decline in milk production at the completion of the experiment should not be interpreted to mean that either of these systems are uniformly superior to good pasture. The brome pasture grazed during the post-experimental period was quite inferior to that grazed during the pre-experimental period.

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CHEMICAL QUALITY, NUTRIENT PRESERVATION, AND FEEDING VALUE OF SILAGES STORED IN BUNKER SILOS

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SUMMARY

Complete input-output weights of four silages combined with chemical analyses and feeding trials were used to determine the performance of bunkers as silage storage structures. Two different silo covers were evaluated with respect to their usefulness for preventing top spoilage. Chopped and unchopped forages also were compared in each of the two experiments.

Chemical quality of the silages was within the range usually obtained in tower silos. Nutrient preservation was less efficient in all silages than that usually observed in tower silos. This lowered efficiency was identified with excessive top spoilage in one experiment, but with high fermentation losses in the other experiment.

The most satisfactory seal was produced with a plastic cover weighted by a continuous layer of sawdust, which markedly reduced the hazard of accidental puncture and helped to maintain the seal during the feeding period. The elimination of surface spoilage in bunker silos by proper sealing techniques and materials appears practicable.

Chopping of forage, although not necessary, was desirable from the standpoint of increasing silo capacity and for convenience in storing and removing of silages. Silage quality and feeding value were improved by chopping relatively dry forage, but no marked improvement resulted from chopping forage of a higher moisture content.

Horizontal storage (in bunkers, trenches, and stacks) of hay-crop silage, while not the predominant method of storage, is assuming a place of increasing importance on the American farm. Such structures are often favorably regarded because of low initial cost. However, a reliable estimate of total storage costs in any type silo requires a knowledge of moisture-free nutrient losses. Useful loss data for horizontal silos are conspicuously lacking, in spite of the abundant literature concerning general aspects of horizontal storage.

Silage losses reported on a fresh weight basis *(6, 7)* are of little value in estimating nutrient loss, because of the variability in silage dry matter content. Cowan *et al. (5)* reported dry matter lost from bags buried in a trench partially covered with a tight plastic. Average dry matter losses were: 31% in uncovered bisulfite-treated silage, 12% in covered bisulfite-treated silage, 30% in uncovered untreated silage, and 23% in covered untreated silage. No losses from direct top spoilage were included in these values, since the bags were all located below the surface area.

Data for comparing the effect of different covering materials on total silo losses also are meager.

A third aspect of horizontal storage requiring additional information is that

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of the practicability of storing unchopped forage in horizontal silos. This modification in usual procedures has been repeatedly demonstrated, and received considerable publicity in U.S. farm magazines since 1950 *(1, 4)*. However, questions concerning the relative merits of chopped and unchopped silage with regard to dry matter preservation, surface spoilage, chemical quality, and feeding value were generally unanswered.

The experiment reported here was initiated to obtain needed information on the following points: *(1)* The efficiency of bunker silos in terms of actual nutrient preservation. *(2)* The advantages and disadvantages of chopping forage used for silage. *(3)* The extent to which plastic covers would prevent top spoilage and nutrient losses in a practical application.

EXPERIMENTAL PROCEDURE

The period of these investigations extended over two crop years, 1954 (Experiment 1) and 1955 (Experiment 2), and the two corresponding winter feeding periods.

Experiment 1, 1954. The second cutting of a mixed crop (mainly orchardgrass and clover) was harvested as chopped and unchopped forage with no intentional wilting and stored in two drive-through bunker silos. The bunkers had concrete floors and tongue-and-groove matched lumber sides lined with asphalt roofing paper. Bunker dimensions were: width 14 to 16 ft., height 8 ft., and length 66 ft. Drainage was provided by a crowned floor that sloped 2 ft. from end to end. Each silo was equipped with two side gutters running the length of the silo and connected by pipe to a seepage-collection pit.

Filling of both silos was started July 6 and was completed in five days with the chopped forage (Silo B-2) and in seven days with the unchopped (Silo B-1). The rather light second cutting was difficult to handle with the buck rakes, thus accounting for the long filling period. The filling rate of the chopped forage was purposely retarded to approximate that of the unchopped. Chopped forage was direct-cut and transported in dump trucks. Unchopped forage was mowed, gathered from the swath with buck rakes, and transported in dump trucks. A wheel-type tractor was employed for continual packing of each silo during the filling period and for one day after filling had been completed. All forage stored was weighed, sampled, and analyzed for chemical constituents.

The seepage from each silo was measured by steam distillate meters placed in the seepage-collection pits. Composite samples of the seepage were analyzed for dry matter content.

The day following final packing, both silos were covered with a plastic sheet constructed from polyvinyl chloride 54 by 0.008 in., joined with pressure-sensitive tape (4-in. width). The plastic was weighted at the edges with pipe and sawdust. No weighting material was used on the central part of the plastic covers. Both silos were opened January 10, following a storage period of about 6 mo. All good silage was weighed as it was removed for feeding, and sampled at five-day intervals. A portion of each sample was analyzed for dry matter, pH, sugar,

carotene, ammoniacal nitrogen, and butyric, acetic, propionic, and lactic acids. The remaining portion of each sample was dried and used in composite samples for proximate analysis. All visible top spoilage was separately weighed, sampled, and analyzed for dry matter content. Analytical procedures conformed to the recommendations of a recent conference of silage investigators *(10)*.

The feeding value of these silages was measured in a 100-day single cross-over feeding trial. The 16 milking cows used in this trial were fed a grain mixture according to milk production (1:4), plus the experimental silage, to the extent of appetite.

Data were obtained on a daily individual-cow basis for weights of feeds fed and refused, and milk produced. Percentage butterfat in the milk was determined for each cow from two-day composite samples taken once during each ten-day period. The dry matter content of silage as removed from the silos was used in calculating the amount of dry matter fed. The dry matter content of refused feed was determined at five-day intervals and that of grain fed determined each 20 days.

Experiment 2, 1955. The first cutting of a grass-clover mixture (mainly orchardgrass) was utilized for the second experiment. Procedures were essentially the same as in Experiment 1, with the following exceptions: Both bunkers were filled during the five-day period, May 6–10, and opened on November 17. Unchopped forage was placed directly in the bunker with buck rakes. The covering material originally planned was not available when the silos were filled, so temporary covers of Sisal Kraft paper (6-ft. width), lapped and weighted with a layer of fresh unchopped grass, were applied May 11. These temporary covers and the weighting forage were removed June 14 and 15 and the permanent covers installed. These covers were home-made from strips of 54-in.-wide neoprene-impregnated nylon with cemented seams. Railroad ties were used for weighting the edges of the covers and the central portion was weighted with a 2–3 in. layer of sawdust.

Samples of good silage were obtained daily during the out-feeding period and held in a refrigerator. These daily samples were composited by ten-day periods and all analyses were made from subsamples of these composites. Fourteen cows were utilized for the feeding trial.

RESULTS AND DISCUSSION

The chemical composition of the chopped and unchopped forages as stored is presented (Table 1). Within-year differences in composition were small, but sizable differences between years were noted with respect to all of the chemical values determined. Differences between years for cutting date, and botanical composition of the crop, are the main factors responsible for the lower dry matter, protein, and carotene, and the higher fiber and sugar values of the forages used in the second experiment.

The chemical analyses of both silages within each experiment (Table 2) were generally much alike. However, in Experiment 1 the chopped forages produced

silage with lower pH, ammoniacal nitrogen, and butyric acid values, and a higher lactic acid value as compared to silage from the unchopped forage. Greater difficulty in consolidation of the unchopped silage, combined with a slow rate of filling, may have delayed the exclusion of air to the extent that the fermentation was affected. Consolidation of both Experiment 2 forages was relatively easy because of a higher moisture content.

The chemical quality of all four silages could be criticized because of relatively high pH, ammoniacal nitrogen, and butyric acid contents, and low lactic acid contents. In these respects, however, they were similar to many silages stored in tower silos without preservatives *(3)*.

TABLE 1

Chemical composition of forages stored

Treatment	Dry matter	Composition of dry matter						
		Protein	Ether extract	Fiber	N-free Extract	Ash	Carotene	Sugar
	(%)						(μ/g)	(%)
Experiment 1, 1954								
Unchopped	29.1	14.3	3.9	27.5	45.1	9.2	215.3	4.8
Chopped	28.7	14.9	4.1	27.5	44.3	9.2	221.6	4.6
Experiment 2, 1955								
Unchopped	22.0	9.8	2.0	30.5	49.7	8.0	168.4	8.0
Chopped	21.8	10.4	2.3	29.5	49.6	8.2	175.3	8.0

The percentages of nutrients (dry matter and dry matter constituents) preserved for feeding are presented (Table 3). Whether or not the forage was chopped, appeared to have little, if any, consistent influence on these values. There was, however, a considerable difference between Experiments 1 and 2 with regard to the preservation rates of some nutrients. Preservation of all constituents except nitrogen-free extract and sugar was greater in Experiment 2 than in Experiment 1. Differences in the physical and chemical characteristics of the initial forage, as well as in covering procedures, could be expected to affect these values.

The total storage loss of dry matter, to which is attached the most economic significance, has been subdivided as visible top spoilage, seepage, and fermentation loss (Table 4). It should be noted that the values for visible top spoilage represent only the dry matter remaining in this layer. It is likely that this layer suffered an additional gaseous loss of dry matter that was not visible, hence accounted for as fermentation loss.

Losses from top spoilage averaged 12.1% in Experiment 1. A layer of spoiled silage 9-11 in. deep occurred on both bunkers immediately under the vinyl plastic covers. These results demonstrated that if plastic covers are not weighted over most of the surface, considerable top spoilage is likely. Small air leaks (along the taped seams in this case) occurring in a cover weighted only at the perimeter permit aeration of most of the surface. Vinyl covering subsequently has been proven satisfactory when overlaid with sawdust.

TABLE 2

Chemical composition of silages as fed

Treatment	Dry matter	pH	Composition of dry matter								Organic acids			
			Protein	Ether extract	Fiber	N-free extract	Ash	Carotene	Sugar	Ammonia N [a]	Butyric	Propionic	Acetic	Lactic
	(%)		(%)					(μ/g)	(%)		(%)			
Experiment 1, 1954														
Unchopped	25.0	5.18	15.4	3.3	28.7	42.0	10.6	173.7	0.4	24.9	2.4	0.4	1.7	1.0
Chopped	24.0	4.77	15.7	3.9	29.8	40.3	10.3	206.3	0.3	16.0	1.6	0.4	2.1	1.8
Experiment 2, 1955														
Unchopped	20.7	4.88	11.4	2.8	36.6	38.8	10.4	169.2	0.2	28.9	4.4	1.6	3.7	0.6
Chopped	19.2	4.90	11.8	2.5	36.1	40.0	9.6	164.1	0.1	29.3	4.3	1.1	3.9	0.5

[a] Expressed as per cent of total nitrogen.

Losses from top spoilage averaged only 3.7% in Experiment 2. Spoilage 2–3 in. deep was present on both bunkers under the Sisal Kraft paper at the time these temporary covers were removed (June 14 and 15) and the permanent covers applied. The paper had suffered marked deterioration during the period of little more than 1 mo. The total amount of top spoilage present when these silos were opened for feeding appeared to be no greater than the amount present when the permanent neoprene-nylon covers were installed. Although the procedure and materials were handicapped in expressing their full value by the delay in application, the average dry matter in top spoilage was only 2.0 lb. per square foot of surface area as compared to 6.7 lb. in Experiment 1.

TABLE 3

Percentage of stored nutrient constituents preserved as good silage

	Dry matter	Protein	Ether extract	Fiber	N-free extract	Ash	Carotene	Sugar
1954 Crop								
Unchopped	71.1	76.5	59.2	74.3	66.3	82.3	57.4	5.2
Chopped	68.7	72.5	64.9	74.4	62.5	76.9	63.9	5.0
1955 Crop								
Unchopped	71.1	82.6	100.3	85.3	55.5	92.4	71.4	1.9
Chopped	72.6	82.5	78.8	88.9	58.6	84.5	68.0	1.0

The success of the covering in Experiment 2 as compared to Experiment 1 was attributed to: (1) Most seam leaks were eliminated. (2) The effects of any air leaks which did occur were confined to a relatively small area by the continuous layer of sawdust. Other work at this station has demonstrated that it is possible to eliminate top spoilage without a weighting material, provided no holes occur in the cover. However, from the practical aspects of limiting the effects of accidental cover injury, and maintaining a seal after one end of the silo is opened for feeding, some continuous weighting material is essential.

The findings of Experiment 1 demonstrated that dry matter losses from top spoilage in a bunker silo which is not effectively covered will be considerably greater than the 3–4% often observed in carefully filled tower silos *(2)*. However, Experiment 2 demonstrated that this loss can be reduced to the tower silo range by proper sealing techniques.

Fermentation losses (Table 4), determined by difference (dry matter stored, less dry matter in seepage, less dry matter in feedable silage, less dry matter in visible top spoilage), include the combined gaseous losses of dry matter from spoilage and feedable silage. Fermentation losses in both experiments would be considered high by tower silo standards. This was the case in Experiment 2, even though a relatively small amount of dry matter remained as surface spoilage. These observations suggest that exposure of silage surfaces to air, particularly early in the storage period, may contribute to total losses to a greater extent than is indicated by the amount of dry matter observed as spoilage. This would explain why high fermentation and low top spoilage losses might occur in a bunker when an air-tight cover is not installed for a month or so following filling. These

TABLE 4
Allocation of losses of stored dry matter

Treatment	Source			Total	Top spoilage
	Top spoilage	Seepage	Fermentation		
	(%)				*(lb. D.M./ sq ft. surface)*
Experiment 1, 1954					
Unchopped	11.9	1.1	15.9	28.9	6.1
Chopped	12.3	0.6	18.4	31.3	7.3
Experiment 2, 1955					
Unchopped	4.2	2.5	22.2	28.9	2.0
Chopped	3.2	3.3	20.9	27.4	1.9

data on fermentation losses also indicate that a determination of only the amounts of top spoilage occurring on horizontal silos may give a distorted picture of comparative ensiling efficiency.

Chopping improved the feeding value of the relatively dry forage (Experiment 1), but no such improvement was noted in the more moist forage of Experiment 2. This relationship was similar to that noted above with respect to chemical quality. The greater daily live weight gains and FCM production obtained from chopped silage (Experiment 1) were highly significant (Table 5). The higher consumption of chopped silage dry matter (per 100 lb. live weight) was not quite statistically significant at the 5% level of probability.

This higher feeding value of the chopped silage (Experiment 1) was probably

TABLE 5
Results of feeding trials

	Experiment 1		Experiment 2	
	Unchopped silage	Chopped silage	Unchopped silage	Chopped silage
Live weight per cow				
Initial *(lb.)*	1,194	1,200	941	946
Average *(lb.)*	1,191	1,216	936	939
Average gain per day *(lb.)*	−0.15[a]	+0.96[a]	−.30	−.48
Milk production (FCM)				
Initial per cow per day *(lb.)*	33.0	33.1	24.6	23.4
Average per cow per day *(lb.)*	30.7[b]	33.5[b]	21.5	21.3
30-day decline *(lb.)*	3.1	0.7	3.6	2.8
(%)	9.5	2.3	14.2	12.0
Feed dry matter consumed per cow per day				
Silage *(lb.)*	21.9	24.4	16.4	16.1
Concentrates *(lb.)*	7.3	7.3	5.1	5.0
Total *(lb.)*	29.2	31.7	21.5	21.1
Feed dry matter consumed per day per 100 lb. live weight				
Silage *(lb.)*	1.84[c]	2.01[c]	1.75	1.71
Concentrates *(lb.)*	0.61	0.60	.54	.53
Total *(lb.)*	2.45	2.61	2.29	2.24
Ratio-grain: milk *(lb.)*	1: 4.2	1: 4.6	1: 4.2	1:4.2
Apparent % TDN in silage dry matter	56.5	69.6	56.8	53.9

[a] Highly significant difference.
[b] Highly significant difference.
[c] f value = 4.46; f value required at 5% level of significance = 4.67.

associated with higher consumption rate. The greater consumption, in turn, may have been associated with differences in chemical quality factors previously mentioned. TDN content, calculated by the differences between average theoretical requirements of the cows and the TDN content of supplements *(8)*, was greater in the chopped silage, although such a difference was not indicated by the proximate analyses. In Experiment 2, when all chemical values obtained on the two silages were about the same and moisture content higher, consumption rates and feeding values were not significantly different.

The practical disadvantages of handling unchopped forage were numerous. Loading the buck rakes from the swath, while relatively simple in Experiment 2, was slow and difficult with the shorter, drier forage in Experiment 1. Time required for filling was considerably greater with the unchopped forage because of relatively small loads, even when the forage was transferred to trucks for hauling. Distributing and packing of the unchopped forage was also more laborious and time-consuming, although this problem was reduced in Experiment 2 by direct placement of small loads in the silo with the buck rake. Vertical cuts in the silage made with an axe or special cutting tool facilitated silage removal, but considerable labor was required, since much of the forage was packed in a tangled position and did not form horizontal layers. Separation of spoilage from good silage also was difficult.

The initial and final densities of the silages are shown (Table 6). An initial density was not determined on unchopped silage in Experiment 1. However, the initial density values determined in Experiment 2 indicate that silo capacity

TABLE 6
Average initial and final densities of silages

	Experiment 1		Experiment 2	
	Chopped	Unchopped	Chopped	Unchopped
	(lb/ft³)			
Initial	42.7	.....	42.4	36.0
Final	49.5	44.5	49.1	49.3

was reduced by using unchopped forage. The final density of unchopped silage in Experiment 1 was less than that observed on the chopped silage; however, the final densities of both silages in Experiment 2 were about equal. This may have been due to the fact that Experiment 1 forages were drier and more difficult to pack. The lower final density of the unchopped forage in Experiment 1 indicates that a greater possibility for the infiltration of air existed in this silo; this may have been responsible for the lower quality observed. The final densities obtained in this experiment approximate the value of 48.8 lb. per cubic foot reported by Otis *(9)* as the average density for a 30-ft. depth of alfalfa silage in a tower silo.

The primary advantage in these experiments of ensiling unchopped forage was that no machinery was necessary for chopping. The additional capital investment required for chopping machinery may be prohibitive, particularly on small farms. This experiment demonstrated that chopping is not essential and that

some of the advantages of ensiling thus may be within the reach of farmers previously excluded because of limited machinery.

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STUDIES ON THE COMPOSITION OF THE RUMINAL FLORA AND FAUNA OF YOUNG CALVES

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SUMMARY

A study was made of the bacteria and protozoa present in the rumen of three calves from 1 to 13 wk. of age, raised under normal conditions. Ciliate protozoa were not established in these calves. A study of older calves in contact with mature animals after 13 wk. of age indicated that entodinia were first established, followed by diplodinia and holotrichs.

Total anaerobic bacterial counts were somewhat higher for the calves than for mature animals. Counts of cellulolytic bacteria were similar to those of a mature animal when calves were 3 wk. old and were quite high even at 1 wk. Lactate-fermenting bacteria were present in high numbers at from 1 to 3 wk. of age, but declined to numbers similar to those of the adult at 9 to 13 wk. Bacteria capable of growth under aerobic conditions and coliforms were highest in numbers when calves were 1 and 3 wk. of age. Even at 13 wk. these counts were higher than those of the adult.

Detailed studies of strains of predominant bacteria revealed 21 different groups that could be grouped on the basis of their occurrence in animals of different ages. The predominant bacteria found in 1- to 3-wk.-old calves were mainly different from those of mature cattle. When calves were 6 wk. of age many groups of bacteria typical of mature animals were found, but several groups not found in mature animals remained. At 9 and 13 wk. of age, bacteria isolated were mainly those typical of mature animals.

That the rumen is physiologically well-adapted for the development of its flora at a very early age is suggested by the following similarities between week-old calves and adults. Most bacteria were nonspore-forming strict anaerobes and higher counts were obtained in rumen fluid agar than in a trypticase-soy agar. Some predominant groups of bacteria were similar, and cellulolytic counts were relatively high in calves, even though milk and straw bedding were the only foods available.

One of the best possibilities for more economical rearing of calves is to obtain rapid development of ruminal function, so that the animal can utilize larger amounts of food of high-fiber content at an earlier age. Ruminal development is evidenced by many changes, including anatomical and physiological changes in the animal and changes in the rumen ingesta, involving kinds of microorganisms and characteristics of the fermentation. Knowledge of the development of the rumen and factors affecting it recently has been reviewed by Flatt *(14)*.

While it is generally believed that microorganisms are necessary for proper rumen function, little is known about the factors involved in the establishment of the kinds of microorganisms necessary to carry on an adequate ruminal fermentation, or the kinds of microorganisms present in the young calf. Pounden and Hibbs *(34)* used direct microscopic methods to study the development of the bacteria and protozoa in young calves. The bacteria studied were selected because of their distinct morphology, which allowed rapid identification. They

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were divided into two main groups, based on their association with predominantly roughage or grain rations. The studies indicated that these bacteria were not present in the rumens of many young calves and that inoculation of calves with rumen contents from mature animals tended to hasten the development of the hay-type bacteria. Heavy consumption of grain slowed the development of the hay-type bacteria. The physiological significance of the bacteria studied was not known.

Huhtanen *et al. (23)* used cultural methods to determine the kinds of bacteria present in the rumen of calves. They found nine types characteristic of calves, which were almost never present in healthy adult cattle on balanced practical rations. Eleven types characteristic of adult cattle were found in calves as early as 2 mo. of age and became more predominant with age. Two types occurred at all ages. Subsequent studies *(15, 21, 22)* indicated that the organisms typical of calves were also typical of adult cattle fed rations high in grain. British workers *(27, 28, 29, 30)* have characterized several different kinds of bacteria isolated from the young calf and goat. The organisms identified included species of coliforms, lactobacilli, streptococci, and gram-negative, facultatively anaerobic, urease-positive rods. More information on numbers present in the rumens of young calves must be obtained before an estimate can be made of their relative importance. However, one species regarded as an anaerobic variety of *Lactobacillus lactis* was found in numbers as high as one billion per gram. An atypical rumen amylolytic streptococcus was found in the rumen of ten-day-old calves and was replaced by typical *Streptococcus bovis* by the time the calf was several weeks old.

The present studies were undertaken to obtain information on the bacteria present in the young calf, raised under the conditions of management of the nutrition and physiology herd at Beltsville, and to determine the age at which organisms typical of mature animals become predominant.

EXPERIMENTAL PROCEDURE

Animals. The three calves used in these experiments included one Holstein (No. 1) and two Jerseys (No. 2 and No. 3). They were taken from the dams within 24 hr. after birth and placed in individual stalls. After about 30 days, they were placed in pens with other calves, but fed individually. They were housed in a barn which contained no mature ruminants, and they had no direct contact with mature animals. They were managed by workers who worked with adult animals also. Colostrum was fed for three days. The Jerseys received 6, 7, 8, 7, 5, and 4 lb. and the Holstein 8, 9, 8, 6, 4, and 2 lb. of pasteurized whole milk for successive ten-day intervals until they were 60 days of age. Beginning at ten days of age, the calves were given free access to good-quality alfalfa hay and a simple, 15.4% crude protein grain mixture.

Sampling and culture procedures. Samples of rumen contents were collected by stomach tube when the calves were 1, 3, 6, 9, and 13 wk. of age. Samples were taken immediately to the laboratory, where the pH was recorded and the sample

diluted for inoculation of culture media as previously described *(5)*. Wet mounts of the sample were observed for ciliate protozoa.

Rumen contents from a mature Holstein cow, fed the same hay and grain as the calves, were studied in the same way as samples from calves. Two samples were collected when the animal was on 10.1 lb. of hay and 5.1 lb. of grain per day. The ration was then changed to 5.4 lb. of hay and 10.1 lb. of grain. After a 2-wk. period, two more samples were studied.

Total anaerobic bacterial colony counts and strains of predominant bacteria were obtained, using the RGCA medium-roll tube method of Bryant and Burkey *(5)*. Colony counts were made in duplicate. Twenty well-isolated colonies were picked at random from the roll tubes and inoculated into RGCA medium slants. With one exception (Calf 3 at 3 wk.), all of these strains (B-strains) came from tubes representing 1×10^{-8} ml. of rumen contents. When growth occurred in the slants, usually after overnight incubation at 37° C., the strains were studied for morphology, using gram-stained smears and wet mounts and a phase-contrast microscope. On the basis of colony type, shape of cells, gram reaction, and motility, representative strains from each sample were selected for further study. Slants of the strains were stored in the dry-ice box to maintain viability until sampling was completed and time was available for detailed studies.

Because of the possibility that many bacteria in young calves would not grow well in the RGCA medium, total anaerobic bacterial colony counts and strains of predominant bacteria also were obtained, using a modified trypticase-soy agar (Baltimore Biological Laboratory). This medium was modified by omitting sodium chloride and adding agar, resazurin, cysteine-HCl, Na_2CO_3, glucose, and cellobiose, as in the RGCA medium. This medium (ATS) was prepared and used with the same procedure used for the RGCA medium, except that strains (T strains) were obtained from representative colonies rather than by picking 20 colonies from each sample, and the strains were maintained in slants of the ATS medium.

Lactate-fermenting bacteria were enumerated, using the lactate-peptone-yeast extract and no-lactate control medium of Gutierrez *(16)*, modified to contain 2% agar so that roll tubes of 18-mm. diameter could be used. These media were incubated for 1 wk. at 37° C. before a colony count was made, and representative colonies were cultured in slants of ATS medium and designated L strains.

Total aerobic bacterial colony counts were obtained in trypticase-soy agar pour plates. Glucose (0.2%) was added to this medium (TS medium). The plates were incubated at 37° C. for 48 hr. before counts were made.

Coliform bacteria were enumerated in violet red–bile agar (Difco Laboratories) pour plates according to the standard procedure. Strains from this medium (V medium) were not isolated for further study.

An estimate of numbers of cellulolytic bacteria was obtained, using the most probable number (MPN) method for coliforms *(34)* but replacing the gas from lactose test with visible disappearance of cellulose in cellulose broth *(5)*. The rumen fluid content of this medium was lowered to 20%. Readings for cellulose

disappearance were recorded after 2 wk. of incubation at 37° C. Most tubes were positive for cellulose digestion after from three to four days of incubation, but occasional tubes became positive between 1 and 2 wk.

Detailed studies of bacterial strains. Organisms selected for detailed studies from the RGCA and ATS media were grouped as gram-positive rods, gram-negative rods, and cocci.

Gram-negative rods and cocci were studied, using media and methods as previously described *(7)*, except as outlined below. Gas production in glucose agar deeps, carbohydrate fermentation studies, final pH in glucose medium, NH_3 production from trypticase, and fermentation acids produced in rumen fluid–glucose medium were those of Bryant *et al. (9)*.

Hydrogen and methane production were determined in the same medium as the fermentation acids. Tubes (18×150 mm.) containing 15 ml. of medium which had been incubated four days at 37° C. were inverted under water and the gas was transferred to the gas-analysis apparatus via a curved glass tube. The gas-analysis apparatus was a semimicro modification of the Newcomer-Haldane constant pressure apparatus. As the medium contained a 100% CO_2 gaseous phase when inoculated, CO_2 production could not be determined. Lactate fermentation was determined in the rumen fluid medium previously described *(4)*. Growth at different temperatures was determined by visible growth in RGCA medium-deep tubes that were stab-inoculated with a loop of culture from the inoculum medium. The agar concentration of the medium was lowered to 0.5%. The cultures were incubated until growth appeared, or for 1 mo.

The studies on gram-positive rods were carried out in the same manner as studies on the gram-negative rods, with the following exceptions: Glucose-cysteine agar slants of Lewis and Rettger *(26)*, modified to contain 0.0001% resazurin, 0.5% glucose, 0.4% Na_2CO_3, and a CO_2 gaseous phase were used. This medium was prepared by the anaerobic technique used to prepare the RGCA medium. The inoculum medium was the above medium minus agar. Ammonia production was determined in the same medium minus agar and with only 0.1% of glucose added. Temperature studies were done using modified glucose-cysteine agar deeps. The basal medium for studies on acid production from carbohydrates was the same medium, modified by omitting agar and glucose and using a 10% CO_2–90% N_2 gas mixture and 0.1% of Na_2CO_3 to lower the buffer capacity. Gas production was determined in the glucose-cysteine medium *(26)*, modified to contain 2% of agar and 1% of glucose. Checks on oxygen relations were made in glucose-cysteine agar shake tubes with 0.5% of glucose and 1% of agar. This medium was prepared with and without 0.1% of filter-sterilized $NaHCO_3$, added when the medium was melted and cooled prior to inoculation. All of the glucose-cysteine media were adjusted, so that the pH was about 6.8 rather than pH 7.4, as used by Lewis and Rettger.

Representatives of lactobacillus groups were studied further to determine other characteristics. Milk medium was the dehydrated product of Baltimore Biological Laboratories, with 0.05% of cysteine-HCl added. It was prepared using N_2 gas and the anaerobic procedure *(5)*. Carbohydrate fermentations were determined in the same manner as for the gram-negative rods and cocci, except

that 1.0% trypticase and 0.5% yeast extract were added to the basal medium in place of rumen fluid and Mineral Solutions 1 and 2.

The medium used to grow certain strains so that the optical rotation of lactic acid produced could be determined was of the following percentage composition: trypticase, 1.0; yeast extract, 0.5; cysteine-HCl, 0.05; $CaCO_3$, 3.0; and glucose, 2.0. The medium was prepared with the anaerobic procedure, using N_2 gas. Inoculum was 2 ml. of a 24-hr. culture of the strain on the same medium into 200 ml. of medium. Incubation was at 37° C. for four days or more, with occasional shaking to insure neutralization of acid with the $CaCO_3$.

The method used to recover the zinc salt of lactic acid was essentially that of Brin, Olson, and Stare *(2)*, except that the culture medium was adjusted to pH 2.0 with concentrated sulfuric acid, filtered, and steam-distilled at approximately constant volume, until volatile fatty acids were removed prior to ether extraction. The isomers of zinc lactate were characterized by optical rotation. Also, the presence of inactive acid was indicated by precipitation of the zinc salt from solution at 37° C. and the water of crystallization was determined for some preparations. Cultures producing known isomers of lactate were included and found to yield the zinc salt of proper rotation. These included *Lactobacillus casei*, L(+)-zinc lactate; *L. leichmannii*, D(−)-zinc lactate, and a heterofermentative lactobacillus which produced racemic lactate.

Strains selected from the lactate agar roll tube cultures were purified by serially diluting cultures in the lactate agar and preparing roll tubes. These were incubated for seven days and well-isolated colonies were transferred back to ATS agar slants, and morphology and motility rechecked. Strains were selected from these for checking lactate fermentation with the liquid lactate and no-lactate control medium, using the method previously described *(4)*, except that total volatile acids were determined rather than the individual volatile acids.

RESULTS AND DISCUSSION

Animals. Table 1 shows the dry feed consumption and weight gains of the experimental calves. The Holstein calf (No. 1) made good weight gains during all periods, except during the first week of life. Calf No. 3 gained in weight until it was taken off milk at 60 days of age. Its feed consumption then dropped off,

TABLE 1

Total dry feed consumption and weight gains of the experimental calves

Age	Calf 1			Calf 2			Calf 3		
	Hay	Grain	Weight gain	Hay	Grain	Weight gain	Hay	Grain	Weight[a] gain
(wk.)	*(lb.)*			*(lb.)*			*(lb.)*		
0– 3	0.9	0.3	−0.8	0.2	0.1	3.6	0.4	0.3	8.2
3– 6	10.8	3.9	22.3	0.7	0.7	11.4	2.9	1.3	15.6
6– 9	25.5	27.4	24.1	14.3	4.3	14.0	9.7	3.3	5.5
9–13	51.4	69.2	31.6	24.6	17.0	−1.1			
0–13	88.6	100.8	77.2	39.8	22.1	27.9			

[a] Birth weights were 92, 55, and 40 lb. for Calves 1, 2, and 3, respectively.

it started to scour, and died at 89 days of age. Calf. No. 2 gained well through 70 days of age. It then lost weight until after 90 days, when it began to make good gains again. The Jersey calves consumed more hay than grain throughout the experimental period, whereas the Holstein calf consumed more grain, especially during the 9- to 13-wk. period.

Data on pH values of rumen content samples did not reveal any significant differences in pH between calves or between ages of calves. Values varied from 6.1 to 7.2, with most samples being around 6.7–6.9.

Protozoa. None of the protozoa, *Entodinium*, *Diplodinium*, or holotrichs were seen in samples from the calves through 13 wk. of age. A few small flagellates were present in all animals. To obtain information on the length of time required for the three groups of ciliate protozoa to become established, samples of rumen content from other calves at various ages were observed. These animals were raised in the same manner as the experimental calves, except that some of them received half of their roughage as alfalfa silage. The calves were transferred to a barn, where they had direct contact with mature animals, when they were 13 to 15 wk. of age. Only entodinia were present in a calf 17 wk. of age and in two calves 20 wk. old. Entodinia and diplodinia were present in two calves 27 wk. old. All three groups, including both large and small holotrichs, were present in animals 33 and 37 wk. old. These observations indicate that calves require rather intimate contact with animals containing the protozoa before the protozoa become established, and suggest a sequence in the establishment of the different groups, e.g., *Entodinium* before *Diplodinium* and *Diplodinium* before holotrichs. It has been shown that by inoculation with rumen contents from mature animals, calves can establish these protozoa at an early age (3 to 6 wk.) *(34)*. Oxford *(31)* stated that *Entodinium* and *Diplodinium* are the first genera to become established, followed by holotrichs.

Culture counts. Data in Tables 2 and 3 show the various culture counts obtained from the calves and from a mature cow. Total anaerobic counts from the RGCA and the ATS media were somewhat higher for the calves than for the mature animal throughout the experimental period. No definite trend due to age of the calves was noted. In most samples, the RGCA medium gave higher counts than the ATS medium. The two samples where this did not occur were at 3 wk. of age and in one of these cases the calf had been treated for scours with bismuth subnitrate.

Counts of cellulolytic bacteria were in the normal range at all ages except at 1 wk. of age. It was rather surprising that these counts were as high as 2.4 million per milliliter at 1 wk. of age. At this time the calves had access to no roughage except the straw bedding. The counts were within the normal range when the calves were but 3 wk. of age, at which time they were consuming little roughage. The rumen contents of the calves at 1 wk. of age were very watery, contained some hair, and were greyish-white in color. Samples from two of the calves (1 and 3) showed a few pieces of straw. These observations indicate that the rumens of week-old calves are well adapted for the establishment of cellulolytic bacteria. The data also indicate that numbers of cellulolytic bacteria in the

TABLE 2

Numbers of bacteria per milliliter of rumen contents obtained by culture counts from young calves

Calf	RGCA Medium				ATS Medium			
	1	2	3	Mean	1	2	3	Mean
Age *(wk.)*	Billions				Billions			
1	2.2	6.3	2.8	3.8	1.7	4.1	1.5	2.4
3[a]	4.8	4.3	0.81	3.3	6.3	3.0	1.0	3.4
6	1.8	1.5	2.1	1.8	1.4	1.4	1.7	1.5
9	3.6	2.1	3.1	2.9	2.2	1.2	0.9	1.4
13	2.4	4.1		3.2	1.8	0.95		1.4
	Cellulose medium				Lactate medium			
Age *(wk.)*	Millions				Millions			
1	2.4	0.3	0.8	1.2	100	950	1,000	680
3[a]	170	54		110	260	210	460	310
6	240	170	540	320	75	55	40	57
9	220	540	540	430	30	12	7	16
13	92	31		62	5.5	5.5		5.5
	TS Medium				V Medium			
Age *(wk.)*	Millions				Thousands			
1	440	1,400	120	650	1,300	30,000	900	11,000
3[a]	64	82	300	150	51	55	81,000	27,000
6	28	48	120	65	32	68	11	37
9	36	55	14	35	100	46	18	55
13	53	39		46	23	19		21

[a] Calf 3 was treated for scours with bismuth subnitrate.

rumen need have little relationship to the amount of cellulose digested or kind of ration fed. The same conclusion could be drawn from earlier studies *(6)*, when it was found that an animal on a ration of grain mixture only (6.3% crude fiber) maintained large numbers of cellulolytic cocci.

Lactate-fermenting bacteria were present in high numbers in the calves at 1 and 3 wk. of age. There was a decline until, at 9 to 13 wk. of age, they were present in numbers similar to those in the mature animal.

Bacteria capable of growth under aerobic conditions and coliforms were present in highest numbers at 1 or 3 wk. of age, and even when calves were 13 wk. of age they had not reached numbers as low as those in the mature animal.

Kinds of predominating bacteria, cocci. Forty-six strains of cocci isolated from

TABLE 3

Numbers of bacteria per milliliter of rumen contents obtained by culture counts from a cow fed rations similar to rations fed calves

Sample	Medium					
	RGCA	ATS	Cellulose	L	TS	V
	(millions)					*(thousands)*
1	800	320	54	14	5.0	2.5
2	730	250	70	23	6.5	4.0
3	1,900	530	110	12	6.6	1.0
4	760	390	170	20	5.4	3.0
Mean	1,000	370	100	17	5.9	2.6

tubes of RGCA and ATS media were selected for study. Thirteen of these were found to be cellulolytic and fit the genus *Ruminococcus* (Sijpesteijn) [Hungate *(24)*] in all characteristics studied. Fermentation products were not determined. These strains were not studied further. Detailed studies made it possible to place 29 of the remaining 33 cultures in five groups (as shown in Table 4). Fermentation products of some representative strains are shown (Table 7).

Group C5 was composed of facultatively anaerobic amylolytic streptococci and were not studied further. It is noteworthy that three of these strains grew at 48° C.

Group C1 was composed of nonmotile, large, lancet-shaped, gram-positive cocci (1–1.2 by 1–2 μ) that formed short to long chains that often included some larger swollen cells. No capsules were seen.

Deep colonies were lenticular and 2–4 mm. in diameter. Surface colonies were 3–5 mm. in diameter, entire, opaque, slightly convex, and white.

Growth in glucose liquid medium was heavily and evenly turbid in 18- to 24-hr. cultures.

Gas production was vigorous, but no H_2 or CH_4 was produced.

These cocci belong in the genus *Peptostreptococcus (1)*, because they are gram-positive, heterotrophic, anaerobic, chain-forming cocci that form abundant gas and produce ammonia from protein hydrolysate. They differ in several characteristics from all recognized species of the genus. They differ from many species in that they do not produce hydrogen or butyric acid, grow over a wide range of temperatures, and ferment many carbohydrates. They appear to be

TABLE 4

Characteristics of groups of noncellulolytic cocci isolated from rumen contents of calves and a mature cow using RGCA and ATS media[a]

Group	C1	C2	C3	C4	C5
Number of strains	4	5	7	9	4
Anaerobic	4	5	7	9	0
Growth at 15 and 22° C.	4	0	0	0	4
Growth at 45° C.	4	5	7	6	4
Growth at 48° C.	0	0	0	0	3
H_2S production	4	5	0	0	0
NH_3 production	4	4	0	0	0
Gelatin liquefaction	0	0	0	7	0
Starch hydrolysis	0	0	0	0	4
Acetylmethylcarbinol	0	0	0	5	2
Gas production	4	5	0	9	0
Final pH glucose	4.5–4.8	5.3–5.9	4.2–4.4	5.1–5.6	4.1–4.3
Acid from xylose	4	0	0	9	0
Acid from arabinose	4	0	0	9	2
Acid from rhamnose	4	0	2	0	0
Acid from maltose	4	5	7	0	4
Acid from sucrose	4	5	5	6	4
Acid from trehalose	4	0	1	0	4
Acid from lactose	4	0	6	7	4
Acid from mannitol	4	4	5	2	3
Acid from glycerol	4	4	0	0	0
Acid from xylan	0	0	0	9	0
Lactate fermentation	0	5	0	0	0

[a] None of the strains produced catalase or indole, or reduced NO_3.

identical with some of the anaerobic streptococci isolated in large numbers from turkey feces by Harrison and Hansen *(19)*. These bacteria produced acid and gas from carbohydrate; fermented glucose, lactose, maltose, trehalose, arabinose, sucrose, and mannitol, and had the very characteristic morphology of the present C1 group. Other characteristics were not determined.

Group C2 was composed of nonmotile, large, lancet-shaped cocci (1.2–1.4 by 1.2–1.6 μ) that usually occurred in two's and as short chains. No capsules were seen. There was some tendency to retain the crystal violet in the gram stain. This was probably due to the size of the cells. However, they were predominantly gram-negative.

Colonies and growth in glucose liquid medium were the same as for group C1.

Gas production was vigorous and included some H_2.

These cocci do not fit any of the genera of cocci recognized in the seventh edition of *Bergey's Manual (1)*, because they are gram-negative and form chains. However, it is probable that they are closely related to organisms now placed in the genus *Peptostreptococcus*. They fit this genus in every respect except the gram reaction. They are similar to *P. lanceolatus* (Prévot) Smith, except that the C2 group produced H_2S and did not hydrolyze starch. *P. lanceolatus* produced acid from starch. This discrepancy could be due to a difference in methods. Lactate fermentation and hydrogen production were not determined. *P. lanceolatus* produced butyric, valeric, and acetic acids in the ratio of 2:1: trace from glucose. The C2 group showed an uptake of acetic and propionic acids and pro-

TABLE 5

Some characteristics of groups of anaerobic, nonmotile, gram-negative rods isolated from rumen contents of calves and a mature cow using RGCA and ATS media[a]

Group	R1			R2	R3		R4	R5
Subgroup	*a*	*b*	*c*		*a*	*b*		
Number of strains	2	3	3	4	5	1	5	19
H_2S production	2	3	3	3	5	1	0	6
NH_3 production	2	0	0	4	5	1	0	7
Indole production	2	2	3	4	0	1	0	0
Gelatin liquefaction	2	0	0	0	0	1	0	11
Growth at 22° C.	1	3	3	1	4	0	0	0
Growth at 45° C.	0	0	0	4	3	0	1	15
Starch hydrolysis	2	3	3	0	0	1	0	14
Cellulose digestion	0	0	0	0	0	0	5	0
Lactate fermentation	0	0	0	4	0	0	0	0
Gas from glucose	0	0	0	4	5	1	0	0
Final pH glucose	5.2	5.2 to 5.3	5.0 to 5.1	5.6 to 6.2	4.7 to 5.6	5.5	5.2 to 6.0	4.8 to 5.5
Acid from rhamnose	0	0	0	0	4	1	0	11
Acid from xylose	0	0	3	0	5	1	0	19
Acid from arabinose	0	0	3	0	5	1	0	19
Acid from sucrose	0	2	2	0	5	1	0	17
Acid from trehalose	0	0	0	0	1	1	0	0
Acid from maltose	2	3	3	4	5	1	0	19
Acid from lactose	0	3	3	0	5	1	0	19
Acid from glycerol	0	0	0	4	5	1	0	0
Acid from mannitol	0	0	0	0	0	1	0	1
Acid from xylan	0	0	3	0			0	19

[a] None of the strains reduced NO_3, grew at 15 or 48° C., or produced catalase.

duction of butyric and/or higher fatty acids in the rumen fluid–glucose medium. The fact that an uptake of propionic acid was observed suggests that part of the butyric and/or higher fatty acid fraction was valeric acid *(12)*.

The C2 group is similar to the LC organism of Elsden *et al. (13)* in every characteristic studied except in the fermentation of sucrose and glycerol, growth at 45° C., and final pH in glucose. However, one of the C2 strains failed to ferment glycerol. The fermentation products recovered from glucose medium appear to be compatible with those of the LC organism. Elsden and Lewis *(12)* showed that propionic and acetic acids were taken up during the fermentation of pyruvate by washed cells of the LC organism.

The RO-C8 organism of Huhtanen and Gall *(22)* also would appear to be closely related to the C2 group. The RO-C8 organism was gram-positive in young cultures but quickly reverted to gram-negative, it fermented lactate and produced acetic and butyric acids and some propionic acid, as well as some higher acids, in the rich organic medium used.

It is probable that the LC, the RO-C8, and the C2 groups would be found to represent one species if studied under comparable conditions, and the possibility exists that they all belong in the species *P. lanceolatus*.

Hobson, Mann, and Oxford *(20)* recently isolated an organism from calves in Scotland that is identical with the C2 group.

Group C3 included nonmotile, lancet-shaped to coccoid bacteria (0.6 μ by 0.6–1.0 μ) that occurred in short to long chains. The cells were often elongated with sharply pointed ends, so that many cells might be considered short rods. They were gram-positive in young cultures. When freshly isolated, many cells of cultures were swollen and quite irregular in shape.

Deep colonies were about 0.5 mm. in diameter and lens-shaped. Surface colonies were about the same size, translucent, entire, convex, and greyish-white in color.

Growth in glucose liquid medium was moderately and evenly turbid in 24 hr.

This group belongs in the genus *Peptostreptococcus (1)*, because it includes gram-positive, anaerobic cocci that occur in chains. It appears to be most closely related to the nongas-forming species, *P. intermedius*. It differs from this species in forming lancets and, often, pointed ends, rather than being spherical, and in forming predominantly lactic acid rather than formic, propionic, and lactic acids. It differs from Group VIa of Hare *et al. (18)*, believed to be similar to *P. intermedius*, in that it usually ferments lactose and mannitol. It has some similarities to the RO-SCC and RO-LCC groups of Huhtanen and Gall *(22)*, but comparisons are difficult because of the few characteristics determined by these workers.

Group C4 included nonmotile cocci (0.6–0.8 μ) that occurred as singles and diplococci and occasionally in short chains. They were weakly gram-positive. They differed from groups C1, 2, and 3, in that there was no tendency toward lancet shapes or pointed ends. No capsules were observed.

Deep colonies were 1–3 mm. in diameter and lenticular. Surface colonies were of similar size, entire, slightly convex, translucent, and greyish-white in color.

Growth in liquid glucose medium was moderately and evenly turbid in 18–24 hr.

Gas production was vigorous and included a substantial amount of H_2.

The C4 group differed from C1, 2, and 3 groups, in that strains were isolated only from the RGCA roll tubes. The other groups were isolated, using both the RGCA and ATS media.

The C4 group fits the genus *Ruminococcus* (Sijpesteijn) Hungate *(24)*, in that they are spherical cells, occur as singles, in two's or in chains, are nonmotile, nonspore-forming, and strictly anaerobic, and ferment carbohydrate with the production of hydrogen and formic and acetic acids. They are most closely related to the species *R. albus (24)*, as no yellow pigment was observed, cells were usually arranged as singles and diplos, and a large amount of hydrogen and no succinic acid were detected. The fact that acetic and formic acids were the main acids produced is in agreement with results obtained on several strains of *R. albus* by Bryant *et al. (10)*. They differ from the genus *Ruminococcus (10, 24, 36)*, in that cellulose is not visibly attacked and they ferment a larger number of carbohydrates than do most strains. However, some authentic cellulolytic *Ruminococcus* strains ferment one or more of the following carbohydrates: glucose, D-xylose, L-arabinose, sucrose, lactose, and maltose. Bryant *et al. (10)* showed that many strains ferment xylan. Mannitol was the only substance fermented by some strains of group C4 which has not been shown to be fermented by authentic strains of *Ruminococcus*. Maltose was the only carbohydrate fermented by some strains of *Ruminococcus*, but not by the C4 group, and this was reported to be a weak fermentation *(24)*. In view of the fact that Hungate *(24)* suggested that noncellulolytic organisms could be included in the genus *Ruminococcus*, it is indicated that the C4 group belongs in the species *Ruminococcus albus*.

According to the seventh edition of *Bergey's Manual (1)*, the *Ruminococcus* genus belongs in the genus *Peptostreptococcus*, because of its shape, gram reaction, relations to oxygen, and cell division in only one plane. However, it does not resemble any of the species now recognized in the genus *Peptostreptococcus*, and this genus contains species with such diverse physiological characteristics that it seems probable that in the future some species will be placed in different genera or subgenera.

Gram-negative rods. Because of the large numbers of strains involved, motile rods were not studied in detail. On the basis of morphology and colony type, most of these bacteria resembled the genus *Butyrivibrio (7)*. A few resembled the following genera of rumen bacteria: *Selenomonas (4)*, *Succinimonas (9)*, *Succinivibrio (8)*, *Lachnospira (8)*, and *Borrelia (3)*.

Fifty-three strains of gram-negative, nonmotile rods isolated from tubes of the RGCA and ATS media were selected for study. Detailed studies made it possible to place 42 of these in five groups (as shown in Tables 5 and 7).

Group R1 was composed of short rods and ovals, 0.8 μ wide, with rounded ends. A few cells were as long as 10 μ and a few chains of cells were always present. They were gram-negative, with a bipolar effect. Subgroup *a* was slightly

TABLE 6

Some characteristics of groups of gram-positive rods isolated from rumen contents of calves and a mature cow using RGCA and ATS media[a]

Group	+R1	+R2	+R3	+R4
Number of strains	3	2	7	9
Oxygen relations[b]	M	A	A	A
Growth at 22° C.	0	0	0	6
Growth at 45° C	0	0	7	8
Gelatin liquefaction	0	2	0	0
Gas from glucose	0	2	0	9
Final pH glucose	5.2	5.1	4.0 to 4.3	4.2 to 4.3
Acid from xylose	3	2	0	0
Acid from arabinose	3	2	1	0
Acid from maltose	3	0	7	9
Acid from sucrose	0	0	7	9
Acid from trehalose	0	0	1	9
Acid from lactose	3	0	7	9
Acid from glycerol	1	0	0	0

[a] None of the strains grew at 15 or 48° C., produced H_2S, NH_3, indole, or catalase, reduced NO_3, or produced acid from rhamnose, mannitol, or xylan.

[b] M = microaerophilic, growth occurred as a zone about 1 cm. below the surface with no growth deeper, or at the surface in glucose agar deeps, when no bicarbonate was present, but grew throughout the medium when bicarbonate was present. A = growth throughout the tube except for approximately the top 5 mm., with no bicarbonate effect.

larger, about 1.0 μ in width, and showed many long cells. Some were up to 30 μ in length.

Deep colonies were lenticular and surface colonies were entire, convex, translucent, and light-buff in color. They varied in size from 0.5 to 3 mm.

TABLE 7

Some fermentation products produced in rumen fluid–glucose medium by bacteria isolated from rumen contents of calves and a mature cow

Strain	Group	Meq. total acid per 100 ml.	Meq. % of total acid						mMH_2 per 100 ml.
			Butyric[a]	Propionic	Acetic	Formic	Succinic	Lactic	
B 43	C1	9.92	0	0	49	3.0	16	33	0
B116	C1	11.36	0	0	50	4.0	15	30	0
B159	C2	1.04	228	−26	−127	35	0	− 6	0.24
T 81	C2	.79	239	−30	−120	38	0	−14	0.15
B 66	C3	4.99	0	0	0	0	0	88	0
B178	C3	5.04	0	0	7	3	0	87	0
B199	C4	7.80	0	0	53	47	0	0	0.50
B210	C4	4.05	0	0	32	73	0	0	0.93
B 40	R1a	11.68	0	25	28	0	42	0	0
B107	R1b	9.24	0	12	25	4	55	0	0
B127	R1c	10.18	0	6	21	3	50	20	0
B 85	R2	1.86	55	8	36	0	0	0	0.12
T 90	R3a	9.06	0	0	8	9	0	83	.....
B 58	R3b	5.99	0	0	13	12	0	66	0.98
B 45	+R1	7.00	0	0	16	20	60	9	0
T162	+R2	8.49	0	0	27	30	0	43	0.41
B180	+R3	10.36	0	0	0	0	0	101	0
T185	+R3	10.16	0	0	0	0	0	103	0
B 62	+R4	9.44	0	0	0	0	0	99	0
T112	+R4	8.34	0	0	0	0	0	100	0

[a] Butyric and longer-chained fatty acids.

Growth in liquid glucose medium was moderately and evenly turbid in 18 to 24 hr. Two strains of Subgroup *c* showed a slimy consistency.

In addition to the carbohydrate fermentation pattern (shown in Table 5), the group fermented dextrin. Subgroup *a* did not ferment galactose, cellobiose, gum arabic, esculin, salicin, or inulin. Subgroup *c* fermented all of these carbohydrates except gum arabic, and Subgroup *b* fermented only the galactose and inulin.

These bacteria belong in the genus *Bacteroides*, as described in *Bergey's Manual (1)* because they are anaerobic, nonspore-forming, gram-negative rods with rounded ends. They are closely related to previously described bacteroides of the rumen, *B. succinogenes* Hungate *(1)*, *B. amylophilus* Hamlin and Hungate *(17)*, and *B. ruminicola* Bryant *et al. (9)*, as they produce succinic and acetic acids and no gas from carbohydrate, do not reduce nitrate or produce catalase, and do not ferment glycerol or mannitol. They differ from all the above species because they grow at 22° C. and produce indole nad some propionic acid.

Group R1-*a* appears to be most closely related to *B. amylophilus*, because it ferments only starch and its hydrolytic products. However, *B. amylophilus* does not ferment glucose and grows well without rumen fluid, in addition to the differences noted above.

Group R1-*c* is closely related to *B. ruminicola* subsp. *ruminicola*. It fits Biotype 2 of this subspecies with the following exceptions: gelatin is not liquefied; less formic acid is produced; indole, propionic, and lactic acid are produced, and growth occurs at 22° C. It differs from all biotypes in the last four characteristics listed.

Group R1-*b* also is most closely related to *B. ruminicola* subsp. *ruminicola*, but differs from all biotypes of this subspecies in growing at 22° C., producing indole and propionic acid, and in its failure to ferment cellobiose, xylose, xylan, and salicin.

It is evident that the succinic acid–producing members of the genus *Bacteroides* present in the rumen can vary considerably in characteristics, making it difficult to place all strains in well-defined species. It is of interest that those found in very young calves differ from all other ruminal bacteroides so far described, in producing indole and propionic acid and in growing at a lower temperature.

Group R2 was composed of short rods, 0.6 μ by 1.2–2.5 μ, with rounded ends and with a few filaments up to 20 μ in length. Some chains were present. It was nonmotile, nonspore-forming, and gram-negative, with a bipole effect. Except for a tendency to be somewhat smaller in width, it was similar in morphology to the R1 strains.

Deep colonies were lenticular and surface colonies were entire, slightly convex, opaque, and white. They were 2–4 mm. in diameter.

Growth in glucose liquid medium was moderately and evenly turbid in 18–24 hr.

Gas production was vigorous and included some H_2.

This group grows equally well in the rumen fluid–glucose and trypticase-yeast extract-glucose media.

This organism belongs in the genus *Bacteroides (1)*, but does not appear to belong in any of the recognized species. It is similar to the rumen species *B. amylogenes* Doetsch *et al. (11)* in fermentation acids produced, but differs greatly from this organism in morphology, carbohydrates fermented, and indole production. It is the only species of bacteroides known to ferment lactic acid. However, many species have not been studied for this characteristic.

Group R3 is composed of rods 0.9–1.2 μ in width at the midpoint of the cell, which tapers off to a sharp point at both ends. Most cells were 2–5 μ long, but cultures contained a few filaments up to 30 μ in length. The cells were nonmotile and gram-negative, with many cells showing a network of deeply staining intracellular material and a few cells showing densely staining granules located near the center of the cell. A few long chains were present.

Growth in glucose liquid medium was evenly turbid in 18–24 hr.

Gas production was vigorous and included a considerable amount of hydrogen.

Growth was equally good in rumen fluid–glucose and typticase-yeast extract-glucose media, and strains were isolated from both the RGCA and ATS roll tubes.

These bacteria belong in the genus *Fusobacterium (1)*, because they are nonspore-forming, anaerobic, gram-negative rods, with definitely pointed ends. However, they differ from any recognized species in producing hydrogen gas. They are similar to some oral strains, Group II of Spaulding and Rettger, in that lactic acid is the main acid and no butyric acid is produced *(25)*. However, they differ from these, in that ammonia and gas are produced and rhamnose, xylose, and glycerol are fermented.

Groups R4 and R5 had morphological and cultural characteristics which were similar to *B. succinogenes (1)* and *B. ruminicola (9)*, respectively.

Gram-positive rods. Thirty strains of gram-positive rods isolated from tubes of RGCA and ATS media were selected for study. Twenty-one of these were placed in four groups (as shown in Tables 6 and 7).

Group +R1 includes nonmotile, nonspore-forming rods 0.4–0.6 μ by 2–4 μ. They were irregularly shaped, with some curved and club-shaped, and a few cells showed branching. Some cells contained granules that were more strongly gram-positive and sometimes these swelled the cell so that they were club-shaped or appeared as short chains of cocci.

Deep colonies were lenticular and 0.5 mm. or smaller in size. Surface colonies were entire, opaque, white, and slightly convex.

Growth in glucose liquid medium was slightly turbid in 48 hr.

This group probably belongs in the genus *Ramibacterium* Prévot *(1)*, as it is a nonmotile, gram-positive rod that shows some branching and ferments carbohydrates. However, it differs in being microaerophilic rather than anaerobic.

It does not fit any of the species described in *Bergey's Manual (1)*, and a search of the literature failed to reveal descriptions of any gram-positive anaerobic or microaerophilic rods that produce a large amount of succinic acid in the fermentation of carbohydrate.

It is similar in some respect to the more anaerobic variety of *Lactobacillus bifidus* [*L. parabifidus*, Weiss and Rettger *(37)*]. +R1 differs from this organism in failing to ferment sucrose. *L. bifidus* has been shown to produce small amounts of succinic and formic acid, along with large amounts of acetic and L (+) lactic acid *(32)*. The fact that the +R1 group produced large amounts of succinic, and small amounts of lactic and acetic acids, might be explained by the presence of the high CO_2 content or other differences in the medium. The difference in growth in glucose agar deeps, with and without added bicarbonate, appears to be in agreement with the studies of Pine and Howell *(33)* on strains of *L. bifidus*. These strains required CO_2 for anaerobic growth and the only strain studied that grew under aerobic conditions required CO_2 for aerobic growth.

Group +R2 was composed of nonmotile, nonspore-forming rods, 0.4–0.6 μ by 0.9–3 μ, with rounded ends. They occurred as singles, pairs, and short chains.

Deep colonies were lenticular and surface colonies were opaque, light-tan in color, entire, slight slightly convex, and 3–4 mm. in diameter.

Growth in glucose liquid medium was heavily and evenly turbid in 18–24 hr.

Gas production was vigorous and included a considerable amount of H_2.

These bacteria belong in the genus *Eubacterium* Prévot *(1)*, as they are gram-positive, nonspore-forming, nonmotile, strictly anaerobic rods that show no branching, long chains, or filaments, but differ from most species in that propionic or butyric acids and ammonia are not produced. While several of these species are known to produce gas, it is not known whether hydrogen is produced. They would appear to be most closely related to *E. biforme* or *E. aerofaciens*. However, they differ from both of these species in arabinose and trehalose fermentation and gelatin liquefaction, and most of the acid produced by these bacteria was nonvolatile *(32)*.

The +R2 group appears to be identical with strains of the +SR-gGXC group previously studied *(5)*.

The +R3 group was composed of gram-positive, nonmotile rods with rounded ends. Cells were often curved and some were club-shaped. A few branched cells were seen. They were arranged as singles and diplos, with a few short chains. There was some variation in size between strains. Some were 0.5–0.7 μ by 1.5–3 μ, with a few cells up to 10 μ long. Others were up to 0.8–1.0 μ by 2–5 μ. Morphological differences other than size were not apparent.

Deep colonies were lenticular. Surface colonies were opaque, white, entire, and slightly convex. They were 4–6 mm. in diameter.

Growth in glucose liquid medium was heavily and evenly turbid or occurred as heavy sediment in 18–24 hr.

In addition to characteristics shown in Tables 6 and 7, Strain B180, representative of the larger strains, and Strains T185 and R62, representative of the smaller strains, had the following characteristics: They produced acid from levulose, galactose, mannose, raffinose, cellobiose, melibiose, salicin, and inulin. They did not produce acid from melizitose, dulcitol, inositol, sorbose or a-methyl-D-glucoside. Only Strain T185 fermented sorbitol. Esculin was split, but not

hippurate. The per cent of acid produced in milk, calculated as lactic acid, was 0.54–0.78. Lactic acid produced was of the laevo-rotatory type.

This group belongs among the homofermentative members of the genus *Lactobacillus*. It differs from many species in producing laevo-rotatory lactic acid. It differs from most species producing this type of acid in being a strict anaerobe and in the fermentation of several carbohydrates. It is similar in many respects to Organism 123 of Mann and Oxford *(29)*, believed to be an anaerobic variety of *L. lactis*, but differs greatly from this organism in morphology; rough-type colonies were never observed, no gas was formed, and inulin was fermented.

This group undoubtedly represents a new species of lactobacillus.

The +R4 group was composed of nonspore-forming, nonmotile, cylindrical rods 0.5–0.7 μ wide by 1.8–60 μ long, with rounded ends. The presence of many long filaments and chains containing both gram-positive and gram-negative elements was characteristic.

Deep colonies were white and lenticular. Surface colonies were entire, white, slightly convex, and translucent-to-opaque. The translucent colonies showed a "frosted glass" appearance. Colonies were 3–5 mm. in diameter. Three strains, including T112, formed surface colonies which had diffuse margins and were opaque. The deep colonies were irregularly lenticular, with diffuse margins.

Growth in liquid glucose medium was heavily and evenly turbid or occurred as heavy sediment at 18–24 hr.

All strains produced a very small amount of gas.

Strains B62 and T112 were shown to have the following additional characteristics: They produced acid from levulose, mannose, galactose, cellobiose, melibiose, raffinose, salicin, and inulin. No acid was produced from melizitose, dulcitol, inositol, sorbitol, sorbose, or *a*-methyl-D-glucoside. Esculin was split, but not hippurate. The amount of acid produced in milk was 0.59–0.68%. The lactic acid produced was laevo-rotatory.

These bacteria are identical with Organism 123 isolated by Mann and Oxford *(29)*, except that inulin was fermented. Organism 123 was regarded as an anaerobic variety of *L. lactis*, as described in *Bergey's Manual (1)*.

Lactate-fermenting bacteria. Many of the strains isolated from the lactate agar were found to be overgrown with contaminants or failed to ferment lactate. This was probably due to the fact that bacteria not fermenting lactate were present in the samples in much larger numbers than were the lactate-fermenters, and that some of the former were able to form relatively large colonies in the lactate medium. This made it impossible to select only lactate-fermenters in the original roll tubes and difficult to determine the numbers of lactate-fermenters in the rumen. The counts of lactate-fermenters (given in Table 2) are only rough estimates, with some bacteria not fermenting lactate included and some lactate-fermenters forming only small colonies probably missed.

Lactate-fermenting L strains included four different groups. One group was identical in morphology and cultural characteristics with the R2 group above. One strain was studied in detail and was found to be identical with the R2 group.

Another group was identical morphologically and culturally with the C2

group. Two of these strains were studied in detail and found to be identical with the C2 group.

A third group was similar to *Selenomonas ruminantium (4)*. One of these was studied in detail and found to be identical with selenomonas strains previously studied, except that H_2S was not produced. In characteristics in which previous strains of selenomonas varied, this organism had the following characteristics: Nitrate was reduced to nitrite; growth occurred at 45° C.; sucrose, trehalose, glycerol, and mannitol were fermented, and starch was not hydrolyzed. Acids produced in rumen fluid–glucose medium included lactate (61%), propionate (23%), and acetate (12%).

A fourth group included gram-positive, nonmotile, nonspore-forming rods 0.7–0.9 μ wide and 1.5–4 μ long. They were mainly cylindrical in shape, with rounded ends, but some cells were curved or unevenly crooked. Some cells were slightly swollen, usually at or near the end, and a few cells showed branching, often with Y shapes. Cells were arranged in singles and in two's, with a few short chains.

Surface colonies in five-day-old lactate agar cultures were 4–5 mm. in diameter, entire, translucent, slightly convex, and light-buff in color.

The following characteristics were determined with Strain L34. Growth in glucose liquid medium was heavily and evenly turbid in 18–24 hr. Final pH was 4.7. Catalase, indole, and acetylmethylcarbinol were not produced, and hydrogen sulfide and ammonia were produced. Gelatin was not liquefied. Nitrate was not reduced. Acid was produced from glucose, sucrose, trehalose, maltose, lactose, and mannitol. Acid was not produced from rhamnose, xylose, arabinose, glycerol, or xylan. Starch and cellulose were not hydrolyzed. Growth occurred at 15 and 45° C., but not at 48° C. It was a strict anaerobe. Acids produced in rumen fluid–glucose medium included acetate (24%), formate (13%), and lactate (59%).

This bacterium belongs in the genus *Ramibacterium* Prévot *(1)*, as it is a strictly anaerobic, gram-positive, nonmotile, nonspore-forming rod that shows some branching and ferments carbohydrates. None of the recognized species in this genus has been studied for lactate fermentation. It has many similarities to *R. ramosum*, but appears to differ, in that lactate acid is produced and it grows through a wider temperature range.

Occurrence of groups of bacteria. Data presented (Table 8) show the numbers of strains of the different groups of predominant bacteria isolated from the animals at different ages. Some of the strains placed in the miscellaneous group undoubtedly belonged in other groups but were lost before detailed studies could be made.

The groups of predominant bacteria can be grouped roughly on the basis of their occurrence in animals of different ages. Those found in young calves and not known to be present in adult cattle include Groups C1, R2, and +R1, which were found only in week-old calves; Groups R1 and R3 found only in 1- and 3-wk.-old calves; Groups C3 and +R4 found only in 3- and 6-wk.-old calves, and +R3, found only in 6- to 13-wk.-old calves. It is possible that one or

TABLE 8

Occurrence of groups of predominant bacteria isolated from rumen contents of young calves and a cow

	Number of strains isolated[a]					
Calf age *(wk.)*	1	3	6	9	13	Adult
Groups of bacteria						
C1	11(1,3)					
C2		6(2,3)	1(1)			
C3		3(1,3)	7(3)			
C4			3(2)	4(2)	1(1)	1(1)
C5	8(2)	5(3)	1(3)			
R1	18(1,2,3)	4(2)				
R2	11(1,2)					
R3	6(2,3)	3(1)				
R4			1(1)	1(3)	1(1)	7(1,2,3,4)
R5			4(2,3)	1(1)	8(1,2)	13(1,2,3,4)
+R1	4(1,2)					
+R2						2(2)
+R3			7(1,3)	1(1)	3(1,2)	
+R4		21(1,3)	4(1,2)			
Ruminococcus		1(1)	3(1,2)	7(2,3)	6(1,2)	5(1,3,4)
Selenomonas-like			3(1)	1(2)		4(1,3)
Butyrivibrio-like	3(1,3)	4(2,3)	12(1,2)	35(1,2,3)	14(1,2)	32(1,2,3,4)
Succinivibrio-like						3(3,4)
Lachnospira-like						2(3,4)
Succinimonas-like						4(3,4)
Borrelia-like					2(1,2)	
Miscellaneous[b]	11(1,2,3)	13(1,2,3)	3(1,2)	11(1,3)	1(2)	8(1,2,3,4)
Total isolates	72	60	49	61	36	81

[a] Numbers in parentheses refer to the calf from which the strains were isolated or, in the case of adult, to the sample number.

[b] The miscellaneous group includes strains that did not fit the other groups and strains from samples in which representatives were lost during storage prior to the detailed study.

more of these groups eventually will be found to be present in mature animals, when these are studied in a wider range of environmental conditions.

A second group includes those groups found only in calves in the present study, but known to be present in mature animals. This includes facultatively anaerobic streptococci (C5 group), which are almost invariably the most numerous bacteria isolated from the rumen that are able to grow under aerobic conditions; the C2 group, of which similar organisms have been isolated from mature cattle by Huhtanen and Gall *(22)* and from sheep by Elsden *et al. (13)*, and *Borrelia*, which is commonly found in mature cattle *(5)*.

A third group includes bacteria found in young calves and adults. Butyrivibrio-like bacteria, ruminococci, noncellulolytic ruminococci (C4 group), *Bacteroides succinogenes* (R4 group), *B. ruminicola* (R5 group), and selenomonas-like bacteria were first found when calves were 1, 3, 6, 6, 6, and 6 wk. old, respectively.

The last group includes bacteria not found in the calves through 13 wk. of age but found in the adult. These include the +R2 group and bacteria similar to *Succinivibrio, Succinimonas,* and *Lachnospira.* As these bacteria are often only a small part of the predominant bacteria found in mature animals, it is quite possible they were present in the calves but were not detected.

The data indicate that the predominant bacteria present in the calf at 1 to

3 wk. of age are mainly different from those of mature cattle, but some groups predominant in mature cattle are present. By the time the calf reaches 6 wk. of age many groups of bacteria typical of mature animals are established, but several groups not common to mature animals remain. At 9 and 13 wk. of age, bacteria isolated are mainly those typical of mature animals.

The four groups of lactate-fermenters were found in the animals of different ages as follows: 1 wk. of age, R2 and C2 groups; 3 wk., R2, C2, *Selenomonas,* and the gram-positive rod group; 6 wk., R2 and C2 groups; 9 wk., R2, C2, and *Selenomonas;* 13 wk., R2, C2, and gram-positive rod group, adult, C2 and *Selenomonas* groups. The main difference between calves and the cow was the failure to find the R2 group in the latter, although it was present in calves through 13 wk. of age.

This study indicates that propionibacteria are not present in substantial numbers in animals at Beltsville. Gutierrez *(16),* however, has found organisms similar to *Propionibacterium acnes* to be present in large numbers in rumen contents and hay, and Huhtanen and Gall *(22)* isolated a similar organism, RO-C1, from the rumen of adult ruminants in several different locations. It seems unlikely that the present cultural conditions were responsible for the failure to isolate these organisms, as strains received from Dr. Gall grew well in both the lactate medium and the RGCA medium.

It is difficult to determine the reasons for the differences in the flora between young calves and adult ruminants, but it is likely that the small amount of dry feed consumed during the first few weeks of life, plus the presence of some milk in the rumen, is of importance. The presence of large numbers of lactose-fermenting lactic acid bacteria (Groups +R3 and 4 and C3) and lactate-fermenters, especially in the first 6 wk., suggests an effect of milk.

Another reason for the differences might be that because the calves were somewhat isolated from mature animals, some kinds of bacteria common to mature animals were unavailable, as indicated by the work of Pounden and Hibbs *(34).*

Along with the differences shown between the flora of very young calves and adults there were many similarities. Most of the bacteria were nonspore-forming strict anaerobes; higher counts were obtained in rumen fluid agar than in trypticase-soy agar; amylolytic streptococci and butyrivibrio-like bacteria were present; succinic acid–forming bacteria that differ only slightly from *B. ruminicola* and *B. amylophilus* were present, and counts of cellulolytic bacteria had reached about a million per milliliter in week-old calves, even though they were eating no appreciable amount of roughage. This indicates that some kinds of rumen bacteria were readily available, and others may have been available but were present in numbers too small to detect.

These similarities between very young calves and mature animals suggest that the rumen is physiologically well-adapted for the development of the ruminal flora at an early age. It may well be that a week-old calf would be able to utilize quite large amounts of roughage if it would consume it.

As the calves contained no ciliate protozoa until after they were 13 wk. old, it is evident that many of the rumen bacteria of mature animals are able to become

predominant without the ciliate protozoa being present. One might speculate that some of the differences in the bacterial flora between the calves, especially at 9 and 13 wk. of age, and mature animals were due to the absence of ciliate protozoa. These differences included somewhat higher total anaerobic, total aerobic, and coliform counts and the failure to isolate certain predominant groups of bacteria from calves that were found in relatively small numbers in the adult.

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DEVELOPMENT OF RUMINATION IN THE YOUNG CALF

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SUMMARY

The activities of 12 Jersey and 14 Holstein calves, with special attention to rumination, were observed for a full day's time each 2 wk. from 1–2 to 15–16 wk. of age. Rumination had begun in 18 of the calves in the first 2 wk., 25 in the second 2 wk., and in all calves in subsequent periods. Rumination time increased rapidly to an average of nearly 5 hr. per day at 6–8 wk. of age. Thereafter, the rate of increase in rumination time was reduced. Rumination time was positively correlated with dry feed consumption, but less time per pound of feed was spent ruminating as the calves aged. The appearance of rumen contents of 17 calves sacrificed at 12–22 days of age indicated that more than half of the calves were ruminating effectively at 2 wk. of age. Observations are also presented of the times spent eating and drinking and lying down, as well as correlations between blood glucose content and rumination time.

The nutritional requirements of the young calf are dependent upon the amount of rumen activity which is attained. Normal mature-type rumen fermentation results in the synthesis of large quantities of B-complex vitamins *(3, 7, 17)*, as well as the formation of amino acids and good-quality protein from ordinary sources *(4, 11)*. Before normal rumen function occurs, the calf secures its needed B-complex vitamins and high-quality protein from milk. After the rumen is functioning adequately, the products of rumen fermentation of ordinary feeds may replace milk in the nutrition of the calf. For greatest economy in calf-rearing, early establishment of, and nutritional dependence on, rumen function are desirable.

The development of the rumen under different dietary regimes has been investigated *(1, 16)*. The development of rumen fermentation and digestion has been shown to approach mature-type function between 6 wk. and 6 mo. of age *(10, 13)*. Rumination is an important adjunct to normal rumen function. Rumination activity in cows has been studied under various conditions *(2, 5)*, but studies of the development of rumination in calves have not been noted. The newborn calf does not ruminate, and calves confined to a sole milk diet do not ruminate. The normal calf, however, soon eats considerable roughage which goes to the rumen *(14)* and, at a relatively early age, begins the process of rumination. Kesler *et al. (8)* stated that rumen contents of all calves 6 wk. old showed evidences of rumination. Because of the importance of rumination in the utilization of coarse and ordinary feeds by the calf, the study reported here was made to chart the development of rumination in young calves.

EXPERIMENTAL PROCEDURE

Calves of the Jersey and Holstein-Friesian breeds from the University of Tennessee herd were the subjects used. The calves were taken from their dams

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at from two to three days of age and placed in individual solid-walled pens, where they remained for 16 wk. Each pen was fitted with containers for water, calf starter, and hay, and these were available to the calf from the first day it was penned. The bedding was oat or wheat straw. A coarse-chopped, alfalfa-grass mixed hay was fed. The concentrate was an 18% protein special pelleted starter formula. Starter consumption was limited to 4 lb. per day per calf. Milk was fed at 10–15% of body weight daily, increasing in amount as the calf grew. After seven days of age reconstituted skimmilk was substituted for whole milk. Jersey calves were weaned at 100 lb. and Holstein calves were weaned at 150 lb. body weight. These weights were reached at an average age of 50.2 days for Jerseys and 43.6 days for Holsteins.

Twenty four–hour observations were made of each of 26 calves at 2-wk. intervals from 1 to 15 wk. of age. The periods of observation were 6 hr. at one time and covered parts of two days, so that the calves were observed all 24 hr. of the day at each biweekly observation period, but not during 24 consecutive hours. For example, the observation times may have been from 1 A.M. to 7 A.M. and 1 P.M. to 7 P.M. on one day, and from 7 A.M. to 1 P.M. and 7 P.M. to 1 A.M. the next day. Calves 5–12 days old were started on the observation schedule, so that in the first observation period calves covering the ages of five days at the beginning to 14 days at the end were included. Subsequent 24-hr. observations were made at 14-day intervals. Each calf was checked at 5-min. intervals during the observation period and it was noted whether the calf was standing or lying and whether it was eating and drinking, or ruminating. Ruminating time included the complete cycles of rumination activity and the short intervals between consecutive cycles. Enough continuous illumination was used in the barn to facilitate accurate observations.

Four calves in the 2- to 3-wk. age range were sacrificed after the 24-hr. observations had been completed. Each of these calves had been fed 2 kg. of reconstituted milk 2 hr. before slaughter. The stomachs and intestines were dissected to observe their characteristics and degree of development. Thirteen other calves in the same age-range, which had been fed different types of milk or milk replacement formulas, were also slaughtered for this purpose.

Blood glucose of 16 Jersey and 16 Holstein calves was determined at weekly intervals from 1 to 16 wk. of age, to compare this measure of changing metabolism in the young calf with the development of rumination. Somogyi's method as adapted by Nelson *(15)* was used. Blood samples were taken 6–7 hr. after the morning feeding.

RESULTS AND DISCUSSION

Early rumination. At the first observation period, eight calves (three Jerseys and five Holsteins) were five to seven days old, and four of the Holsteins were observed ruminating; eight calves (three Jerseys and five Holsteins) were eight to ten days old, of which five (two Jerseys and three Holsteins) were observed ruminating; and ten calves (six Jerseys and four Holsteins) were 11 to 14 days old, of which all but one Jersey were ruminating. Two weeks later (19 to 28 days old) all of the Holsteins were ruminating, as well as 11 of the 12 Jerseys.

At the third period (33 to 42 days old) all calves were ruminating regularly and at length. These observations indicate that the rumination process begins at a very early age in hand-fed calves and that most calves begin ruminating at about 2 wk. of age.

Early rumen-reticulum contents. The appearances of the fore-stomach contents of four calves which were sacrificed after 24-hr. observation periods are given (Table 1.) The appearance of rumen contents from ruminating calves

TABLE 1

Appearance of rumen contents upon slaughter 2 hr. after feeding, from calves which had been observed for rumination activity for the previous 24 hr.

Calf	Age	Ruminating periods	Total time ruminating	Weight of rumen contents	Weight of rumen liquid	Rumen content appearance
	(days)	*(No.)*	*(min.)*	*(g.)*	*(g.)*	
774	12–14	0	0	1004	876	Coarse hay and straw (up to 2 in.), some grain, much liquid
B787	12–14	8	100	766	—[b]	Finely macerated, mushy mixture of hay, grain, straw, and hair
B792[a]	16–18	11	90	1147	282	Finely macerated, mushy mixture of hay and grain
T474	15–17	11	210	1364	—[b]	Finely macerated, frothy, mushy mixture of hay, grain, hair, and shavings

[a] B792 was observed for 24 hr., also at ten days of age, and was not ruminating.
[b] Liquid from rumen contents would not drain through cheese cloth.

was markedly different from that of a nonruminating calf. Also, the characteristic sharp, acid rumen odor was noted from all ruminated contents, whereas it was lacking from the nonruminating calf. Rumen contents of 13 other Jersey calves, 12 to 22 days old, were examined in a similar manner. Based upon appearance, eight of these had been ruminating effectively and five (all less than 15 days old) had been ruminating little or not at all. In the nonruminating calves, the abomasum contents averaged 2,202 g., but the rumen contents only 809 g. In the ruminating calves, the organ contents were nearly equal, averaging 1,389 g. in the abomasum and 1,064 g. in the rumen. Feed material was rarely observed in the omasum of these young calves.

From the amount and appearance of stomach contents, the indication was that rumination accompanied or preceded the development of normal rumen fermentation and function. The nonruminated rumen contents frequently had a foul odor, the liquid and solid materials were easily separated, and no gas retention was noted. Rumen contents were larger in amount in the ruminating calves, indicating that greater amounts of feed were being channeled through the rumen; and a uniform feed-liquid mixture containing gas showed that active fermentation was present.

Number and length of ruminating periods. The average number of ruminating periods per day, and the average time of each ruminating period at the

eight biweekly observation periods, are given (Table 2). These data show that there was a progressive increase in both number and length of ruminating times through the first three observation periods (to about 6 wk. of age). After this time, the change in these factors was gradual, with the maximum number of times observed at the last age period. Increasing daily rumination time was due mainly to more frequent ruminations as the calves aged.

Daily ruminating, eating, and resting time. The average total times per day spent in various activities according to the eight age periods are presented (Table 3). The average time spent ruminating at the first observation period

TABLE 2

Average number of ruminating periods and length of each period in Jersey and Holstein calves up to 16 wk. of age

Age at observation periods	Jerseys (12 calves)		Holstein (14 calves)		All calves	
	Periods per day	Av. time per period	Periods per day	Av. time per period	Periods per day	Av. time per period
(days)	*(No.)*	*(min.)*	*(No.)*	*(min.)*	*(No.)*	*(min.)*
5–14	1.7	17.0	4.4	14.6	3.1	15.2
19–28	8.7	20.1	9.9	19.6	9.3	19.9
33–42	12.8	23.4	11.9	23.1	12.3	23.2
47–56	11.5	26.0	11.8	24.8	11.7	25.3
61–70	11.1	23.2	12.7	26.0	12.0	24.8
75–84	11.7	24.8	13.1	27.1	12.5	26.1
89–98	12.6	25.3	14.9	24.4	13.8	24.8
103–112	15.0	23.6	15.5	25.3	15.3	24.6

was less than 1 hr., for all calves, and the variability was large, as evidenced by a standard error of the mean of 14 min. Total rumination time increased to about 3 hr. the next 2 wk. and to nearly 5 hr. by 6 wk. of age. Thereafter, the rate of increase with advancing age was slow. In general, the variability in rumination time decreased as the calves grew older. The times spent ruminating by calves over 6 wk. of age approached similar values reported for lactating cows or heifers, on feed of similar roughage content *(2, 5)*.

The average times spent eating and drinking were related to the types of feed the calves received. Nearly all of the calves were receiving liquid milk for the first 6 wk. (three observation periods) and this was consumed rapidly. Important intakes of hay and grain also started at about the 5th wk., as shown in Table 4. As dry feed consumption increased, eating time increased to a maximum of about 4 hr. at about 10 wk. of age. After this time, eating efficiency increased, because as feed intake continued to rise less time was spent in eating.

The calves spent most of their time lying down. They averaged 17.5 hr. lying in the first period. This time gradually decreased to 14.9 hr. per day in the third period. After this age, there was little change in the degree of this activity through 16 wk. of age.

Correlation of rumination and feed consumption. The average consumption of milk, hay, and grain by the two breeds of calves is presented by observation periods (Table 4). Two of the Holstein calves in this summary were observed

TABLE 3

Average times in 24 hr. spent ruminating, eating, and drinking, and lying down by 12 Jersey and 16 Holstein calves up to 16 wk. of age

Average age when observed	Characteristic	Jerseys			Holsteins			All calves		
		Ruminating	Eating and drinking	Lying down	Ruminating	Eating and drinking	Lying down	Ruminating	Eating and drinking	Lying down
(days)		*(min.)*								
9	Av.	28	102	1,024	64	94	1,075	47	98	1,052
	S. E.[a]	11	10	47	24	10	30	14	7	27
23	Av.	175	156	978	194	166	998	185	162	988
	S. E.	23	17	41	22	10	25	16	9	23
37	Av.	300	190	894	274	204	891	286	197	892
	S. E.	20	18	28	22	17	25	14	11	19
51	Av.	299	183	903	292	216	922	295	201	913
	S. E.	25	13	30	27	18	25	18	11	19
65	Av.	258	235	872	331	260	838	297	248	854
	S. E.	24	17	40	14	14	25	15	11	22
79	Av.	289	230	888	356	246	872	325	238	879
	S. E.	12	17	25	17	19	31	12	13	20
93	Av.	319	220	886	362	241	857	342	231	870
	S. E.	31	13	27	16	11	23	17	8	18
107	Av.	354	199	908	393	232	874	375	217	890
	S. E.	19	13	17	10	15	22	11	10	14

[a] S. E. is standard error of the mean.

TABLE 4

Average daily consumption of milk, hay, and grain (starter) by Jersey and Holstein calves observed for rumination activity

Age	Jerseys (12 calves)			Holsteins (16 calves)		
	Milk	Hay	Grain	Milk	Hay	Grain
(wk.)	(lb.)					
1–2	5.95	0.04	0.06	9.23	0.06	0.10
3–4	6.87	0.08	0.35	10.47	0.17	0.70
5–6	7.48	0.33	0.80	9.08	0.47	1.67
7–8	5.24	0.48	1.55	4.98	0.87	3.22
9–10		0.65	2.47		1.60	3.71
11–12		0.90	2.72		2.67	3.90
13–14		1.18	3.41		3.63	3.95
15–16		1.89	3.69		4.32	3.97

only during the last four periods. The dry feed intake data are plotted against the corresponding average ruminating times of the same calves in Figure 1. This graph shows a close correlation between feed intake and ruminating time, with slight differences between the breeds. Linear correlation coefficients (r) calculated from the individual paired observations of ruminating time and weekly feed intake were 0.62 for Jerseys, 0.75 for Holsteins, and 0.69 for all calves over the whole range of observations. When the Jersey data for the first

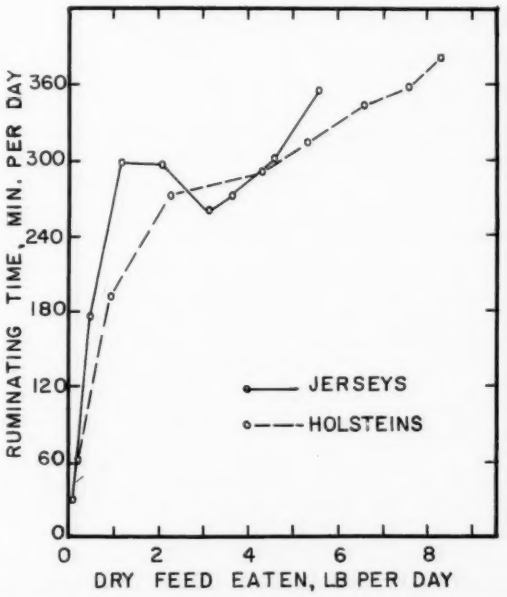

FIG. 1. Relationship between daily average consumption of hay and grain and the observed rumination times of Jersey and Holstein calves at biweekly intervals.

three periods and the Holstein data for the first two periods were omitted, the correlation coefficient for all calves was 0.51. All of these values indicate a highly significant correlation between rumination time and feed intake. This is in full agreement with similar observations reported on older cattle *(5)*.

One interesting feature of this study was the indication of rumination practice in the very young calves. During the first three periods, while the calves were subsisting primarily on milk, rumination activity developed rapidly, although dry feed intake averaged only slightly over 1 lb. per day. Up to 5 hr. were spent ruminating with this small amount of feed, most of which was grain. After these periods, much larger amounts of feed were consumed, with little increase in ruminating time. This would indicate that the development of normal rumen function is a usual precedent for the calf to be able to handle large amounts of dry feed properly.

Blood glucose and rumination time. Average blood glucose values taken at weekly intervals are compared with the biweekly average rumination times in Figure 2. It is shown that blood glucose decreased for the first 8 wk., after which it remained relatively constant. Rumination time followed the same pattern inversely up to 8 wk. and thereafter continued to increase at a reduced rate. There was a highly significant negative correlation ($r = -0.80$) between the grouped average blood glucose and rumination times. The drop in blood glucose in young calves has been explained as due to a change in rumen digestion *(6, 9, 12, 13)*. It is, therefore, indicated that adult type rumen function, both mechanical and biochemical, is essentially achieved in calves fed, as these were, by 5–6 wk. of age.

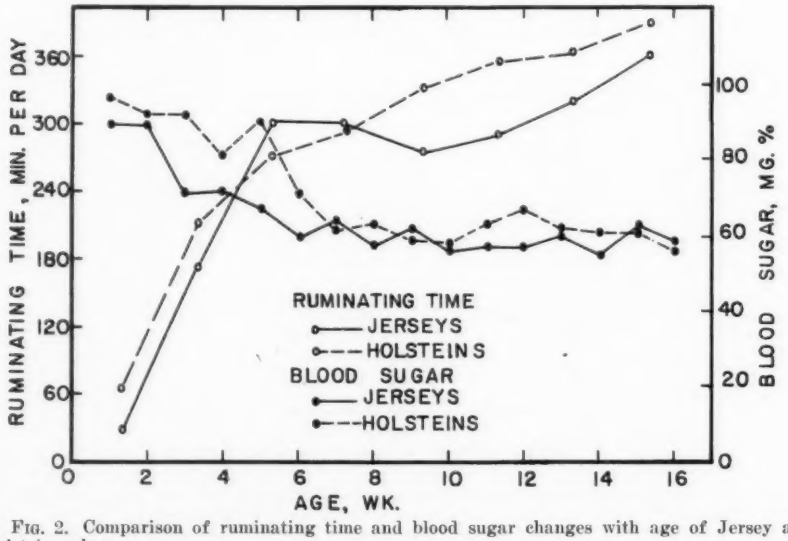

FIG. 2. Comparison of ruminating time and blood sugar changes with age of Jersey and Holstein calves.

Other observations. Diarrhea in two calves was observed to markedly reduce rumination. One scouring calf ruminated only 15 min. in the sixth period, compared to 305 min. in the fifth period, when it was in normal condition. Another calf dropped from ruminating 285 min. in a normal sixth-period observation to 115 min. in the seventh observation period when afflicted with mild diarrhea. Only very slight reductions in daily feed consumption accompanied the diarrhea attacks of these calves; so, the reduced rumination probably resulted in considerable change in the character of the contents of the gastrointestinal tract. These were the only instances in this study in which diarrhea was noted. Two calves, not included in this study, were observed which had respiratory illness at 2–3 wk. of age. Neither of these calves was ruminating, probably as a result of the early age and sickness combined.

Because of the high incidence of calf sickness on dairy farms and of the widely varying methods of feeding and rearing calves, it would be misleading to state any narrow range of ages as that at which effective rumination is dependably established. These studies indicate that such an age would be between 2 and 4 wk. This may not be true for calves fed heavily on milk or for unhealthy calves.

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COMPARATIVE UTILIZATION OF ALFALFA-BROMEGRASS PASTURE UNDER ROTATIONAL AND DAILY STRIP GRAZING [1]

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SUMMARY

The efficiency of herbage utilization from alfalfa-bromegrass (*M. falcata* and *B. inermis*) pasture under rotational and strip-grazing management was compared for three grazing seasons. The expression of increased efficiency as increased carrying capacity was precluded by restricting grazing, so that the cows on each system had equal opportunity to utilize the available herbage. Under these restrictions, dairy cows at a moderate level of milk production utilized alfalfa-bromegrass pasture almost as efficiently under rotational grazing as under strip grazing.

Herbage utilization has been called the chief problem of grassland management *(5)*. *Medicago falcata* is presently the only winter-hardy alfalfa available for use in interior Alaska, and *Bromus inermis* is a highly productive grass under Alaskan conditions. This study is a continuation of investigations being carried out to determine the effectiveness of increasing intensities of pasture management or the efficiency of herbage utilization.

Reports in the literature suggest that increased efficiency of herbage utilization is expressed as increased carrying capacity per acre, with little or no apparent effect on the level of production maintained by the individual animals. Brundage and Petersen *(1)* and Davis and Pratt *(4)*, comparing continuous grazing with strip and rotational grazing, respectively, found that the intensity of grazing control did not affect animal production significantly.

Controlled grazing resulted in more efficient herbage utilization, however, because it provided for salvage of material in excess of grazing needs. Ittner *et al. (10)* and Meyer *et al. (13)* found insignificant differences in rate of gain for beef steers rotationally grazing, strip grazing, or soiling alfalfa. The total yield of beef per acre was highest under the soiling system *(9, 10, 13)*. Total production of beef was comparable under rotational and strip grazing when periods on the rotated paddocks were limited to from five to seven days. When the time on each paddock was increased to from eight to 12 days, production was higher under strip grazing. Holmes *et al. (6, 7)* obtained 20–40% increases in production per acre when cows were close-folded, compared to rotational grazing. They attributed these increases to increased efficiency of utilization. Hosking and Line *(8)* found that daily herbage allocations gave 15% more economical use of available pasture than did four-day rotational grazing. They found no significant differences in milk yield, live weight changes, or dry matter intakes which could be attributed to the grazing systems.

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Brundage and Sweetman *(2)* obtained 63% greater production from mature oats and peas pasture when it was offered in twice-daily breaks, compared with uncontrolled access. Controlled grazing provided increased carrying capacity with no increase in daily milk production per cow. Procter and Hood *(14)* obtained 25% greater utilization of herbage with close-folding, compared with intensive rotational grazing. Restricting grazing so that each group had equal access to the available herbage, Brundage *et al. (3)* found little difference in animal production or efficiency of utilization of a moderately productive bromegrass pasture rotationally or strip grazed. Ivins *et al. (11)* have recently suggested that often the potential production of the grazing animals, rather than of the pasture, is the limiting factor, regardless of the pasture type or treatment. They stressed the importance of dry matter yields supplemented with palatability and digestibility data.

In the experiments discussed above, increased efficiency of pasture utilization has been obtained through increased carrying capacity of the sward, with little or no increase in individual level of production. It is conceivable, however, that more intensive utilization of grassland might be expressed in higher levels of production per grazing animal, with no increase in the number of animals maintained. The experiment to be described in this paper was designed to maintain equal carrying capacity under two management systems, rotational and strip grazing. Any difference in efficiency of herbage utilization must be expressed in terms of increased production per animal unit.

EXPERIMENTAL PROCEDURE

Seven acres of alfalfa-bromegrass pasture were used in 1955, 1956, and 1957 to compare the efficiency of herbage utilization under rotational and strip grazing. The field was divided down the long axis into two equal areas. One area was further subdivided into three equal paddocks and rotationally grazed. The other was daily strip grazed, using portable electric fences *(3)*. Grazing under both systems was restricted, so that each group had equal opportunity to utilize the available herbage. It was necessary to remove both groups at intervals, to allow sufficient recovery of the pasture for further grazing. Management systems remained the same for each area during all 3 yr.

Different groups of eight milking dairy cows were matched into four pairs at the initiation of grazing each year. One member of each pair was assigned at random to one management system, the other cow going to the second system. They remained under the assigned management programs for the entire grazing season. One pair was removed from the experiment at the completion of the second grazing cycle in 1956, because of different expectant calving dates.

The cows were weighed prior to leaving the barn for pasture after the morning milking. They were allowed water from a common trough after weighing, thereby reducing the variable of water fill in the weight data. Weights taken at the beginning and end of each complete grazing cycle (once over the pasture) were used to compute weight changes and maintenance requirements. Milk production

was recorded twice daily and sampled at ten-day intervals for butterfat tests. A limited quantity of grain was given one pair of cows in 1955. None of the other animals received grain supplementation at any time while on experiment. These data were used to compute TDN utilization according to the procedure of Knott *et al. (12)*.

RESULTS AND DISCUSSION

Animal production, the only measure of relative herbage utilization under the two management systems, is summarized (Table 1). Each system supported the same number of actual cow-days on pasture because of the restriction in design imposed. TDN production per acre was higher each year under strip grazing, but differences were small the last 2 yr. of the experiment. The large differences evident in 1955 reflect milk production differences for one pair of cows. Although

TABLE 1

Production from alfalfa-bromegrass pasture grazed 3 yr. under the same two management systems

	1955		1956		1957	
	Rotational grazing	Strip grazing	Rotational grazing	Strip grazing	Rotational grazing	Strip grazing
Number of cows	4	4	4[a]	4[a]	4	4
Acres	3.6	3.45	3.6	3.45	3.6	3.45
Number of grazing cycles	4	4	4	4	3	3
Days on pasture	75	75	91	91	69	69
Average daily production per cow						
FCM *(lb.)*	28.10	33.18	29.06	27.72	26.05	24.27
Weight gain *(lb.)*	0.78	1.46	1.58	1.49	0.49	0.72
Weight loss *(lb.)*	0.33	0.14	.....	.....	0.06	0.15
Average daily grain ration per cow *(lb.)*[b]	5.1	5.1	.....	.....	.....	.....
Production per acre from pasture[c]						
TDN *(lb.)*	1,622	2,061	2,094	2,198	1,498	1,546
Standard cow-days	101	129	131	137	94	97

[a] One cow under each system was removed at the completion of the second grazing (48 days).
[b] One cow under each system was fed grain (67.3% TDN) the first year.
[c] Calculated according to the method of Knott et al. *(12)*.

nearly equal in level of production at the start of the experiment, the cow rotationally grazing failed to maintain production at a level comparable to its mate. Whether this is a reflection on the management system or on the cow involved is not definitely known. The lowered TDN production of the pasture in 1957 was the result of a severe and prolonged drought that spring and early summer.

Daily milk production was more uniform under strip grazing. Milk production was cyclic on the rotated pasture, rising when a new paddock was entered and falling as the available herbage was depleted through consumption and fouling. Even though the paddocks often appeared severely depleted before the animals were moved, total production was comparable or superior to that on the more uniform regime. The time spent on any one paddock varied from four to nine days.

Therefore, these data fail to show a consistently large difference in efficiency of herbage utilization between rotational and strip grazing. But caution must be used in deriving general statements from these results. The rotated animals were often required to remain on paddocks which were seriously depleted, to satisfy the requirement that each group be given the same opportunity to graze comparable areas. Although good herdsmanship would demand their rotation at these times, they were able to recover when moved to a new paddock and maintained their total production at a level comparable to the strip-grazed group. As Ivins *et al. (11)* suggested, the potential productive level of the grazing animals, and not the pasture, may have been a limiting factor. The rotation interval (from four to nine days) may have been too short to deplete the animals sufficiently before they moved to a new paddock. Finally, the productive potential of the pasture may have been such that a good rotation system was nearly as efficient as strip grazing. Efficiency of utilization is a function of the potential producing ability of the grazing animals and the pasture being grazed. As the level of either or both rises, more intensive grazing may be necessary to maintain a comparable level of efficiency.

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NUMBER OF SPERMATOZOA IN THE DIFFERENT PARTS OF THE REPRODUCTIVE TRACT OF THE BULL[1]

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SUMMARY

A study was made of the distribution of spermatozoa in the reproductive tract of the bull. The study was subdivided into three separate phases. In the first phase, the sperm numbers in the ampulla, ductus deferens, and the cauda epididymis were determined. The average values were as follows: *(1)* 22 ampullae, 418 million, *(2)* 10 ducta deferentia, 340 million, *(3)* 25 cauda epididymides, 8,806 million. The number of spermatozoa in the ampullae and the ducta deferentia of a bull is not sufficient for a normal ejaculate, whereas the cauda contains enough spermatozoa for a number of ejaculates.

During the second phase, the distribution of spermatozoa within the epididymides of 46 bulls was determined. The cauda epididymis contained 45.42%, corpus, 18.34%, and the caput, 36.24% of the total spermatozoa counted in the epididymides.

The relation of testicular and epididymal weights to the sperm contents in the cauda and caput epididymis was studied in the last phase. The correlation coefficients between the following variables were calculated on a between-bull basis: *(1)* testicle weight and sperm number in the cauda, 0.16, *(2)* epididymis weight and sperm in the cauda, 0.32, *(3)* cauda weight and sperm in the cauda, 0.37, *(4)* caput weight and sperm in the cauda, 0.36, *(5)* testicle weight and sperm number in the caput, 0.20, *(6)* epididymis weight and sperm in the caput, 0.27, *(7)* caput weight and sperm in the caput, 0.34. None of the correlation coefficients was significant.

The average number of spermatozoa contained in an ejaculate of a bull is between four and five billions *(1, 9)*. Ejaculates with numbers of spermatozoa that greatly exceed these values are frequently observed. Individual workers *(1, 8)* have stated that the epididymis serves as the storehouse for spermatozoa. No statements have been found in the literature concerning the immediate source of spermatozoa in an ejaculate.

Kirillov in 1937 *(6)* and Gassner in 1953 *(4)* reported a profound diminution in the number of spermatozoa in a bull's ejaculate following vasectomy. Actual counts of the sperm contents of the reproductive tract were reported by Poloveeva *(12)*. In two castrated rams the epididymides contained on the average 162×10^9 spermatozoa, 76% of which were found in the cauda. Chang *(2)* found 130×10^9 sperm in the epididymides of the ram, of which 79% were contained in the cauda, and the ampullae contained only 1.4% of the spermatozoa. Ortavant *(11)* reports the following values for the cauda epididymis of the bull and ram, respectively: $15\text{–}20 \times 10^9$ and $40\text{–}60 \times 10^9$ spermatozoa. According to Rao and Hart *(13)*, the concentrations of spermatozoa in the ampullae and the cauda epididymides were $1{,}240\text{–}4{,}510 \times 10^3/\text{mm}^3$ and $2{,}020\text{–}9{,}310 \times 10^3/\text{mm}^3$, respectively. The volumes of fluids obtained were not stated.

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This study was undertaken to clarify the question concerning the source of spermatozoa for ejaculation in the bull.

EXPERIMENTAL PROCEDURE

The material used in this study was obtained from 25 dairy bulls slaughtered at the local meat-packing plant. All of the bulls are not represented in each phase of the study. The reproductive tracts were removed within 30 to 45 min. after slaughter. The material was taken to the laboratory and processed as rapidly as possible.

In Phase I of this study, the total numbers of spermatozoa in the cauda epididymis, ductus deferens, and ampulla were determined. The spermatozoa from these three sections of the reproductive tract were obtained by flushing out with isotonic sodium citrate delivered under pressure from a syringe. In the case of the ampulla and the ductus deferens, this was an easy task. With the cauda epididymis, care had to be exercised to avoid producing a rupture by excessive fluid pressure. Flushing was continued until only a few cells were being recovered in the fluid. A red blood cell dilution pipette and the improved Spencer brightline hemocytometer were used for the estimation of sperm concentration in the flushings. Cells in both chambers were counted, and if the difference between the two values was not more than 10%, the values were averaged. A new count was made whenever the difference in values was greater than 10%. The total number of spermatozoa in a sample was calculated according to the following formula:

$$\text{Total number} = \text{average hemocytometer count} \times \text{pipette dilution rate} \times \text{volume of flushings} \times 10^4$$

In Phase II of this study, the relative distribution counts of spermatozoa in the caput, corpus, and cauda epididymis were made. Each epididymis was sectioned into the three components. Since it was extremely difficult to cannulate the tubules in the caput and the corpus, and also because the cut went through the epididymal tubule in a number of places, it was found necessary to macerate the tissues. The caput, corpus, and the cauda were sectioned into small pieces, then all of the pieces from each component were macerated in a Waring blender in about 75 ml. of a sodium citrate solution. The blending was carried out for approximately 10 min., or until the whole mass of tissue was reduced to a somewhat homogeneous mass. Due to the presence of a larger amount of connective tissue in the caput, it was often found necessary to repeat the maceration. Tissue debris in the samples precluded the use of the red blood cell dilution pipette. Each sample was diluted 1:10, then it was shaken well, and a sample was introduced into the hemocytometer for counting. The presence of excessive sperm breakage and tissue debris necessitated the counting of sperm heads only. The disintegration of sperm heads by the maceration procedure is not likely. The total number of spermatozoa was determined by the following formula:

$$\text{Total number} = \text{average hemocytometer count} \times \text{dilution rate } (1:10) \times \text{volume of sample} \times 10^4$$

In Phase III of this study, the weights of the following parts were recorded: testicle (without tunica vaginalis and epididymis), epididymis, cauda epididymis, and the caput epididymis. The sperm numbers in the caput and the cauda epididymis were determined from macerated samples, as in the preceding phase.

With the exception of the last phase, where both sides of the reproductive tract were used, only one side of the tract was used. The side used was chosen at random. In a number of cases only one side was available, because of damage through processing at the slaughter-house.

RESULTS AND DISCUSSION

Phase I. Table 1 gives the average numbers and variation of spermatozoa contained in the ampulla, ductus deferens, and cauda epididymis. The variation among samples as to the number of spermatozoa contained was very pronounced. Part of the variation could be caused by the sexual activity of the bulls prior to slaughter. These animals were confined to a common pen and were observed

TABLE 1

The average total numbers of spermatozoa in the ampulla, ductus deferens, and cauda epididymis of the bull

	Ampulla	Ductus deferens	Cauda epididymis
No. samples	22	10	25
No. spermatozoa ($\times 10^6$)	418	340	8,806
Standard deviation ($\times 10^6$)	368	208	5,933

to ride one another. The method of slaughtering did not produce an ejaculatory response, for the examination of the urethras did not reveal abnormal numbers of spermatozoa; the penises were generally in a retracted state. In this table, the value for the cauda epididymis includes only the samples obtained by flushing out of the caudas. It was felt that the data from the macerated samples were subject to greater counting error because of the damaged spermatozoa and cellular debris.

It is apparent (from Table 1) that the ampulla and the ductus deferens do not contain enough spermatozoa to form a normal ejaculate. The cauda epididymis with nearly nine billion spermatozoa contains enough cells for a number of ejaculates *(15, 16)*. The value for the cauda, as reported in this paper, is considerably lower than the 15 to 20 billion reported by Ortavant *(11)*. Bull differences could account for this difference, for among the 25 samples there were seven that were in the range of, or even higher than, the values given by Ortavant.

It is a well-established fact that increased scrotal temperatures are injurious to spermatogenesis and spermatozoa. For this reason Kirillov *(6)* suggested that parts of the reproductive tract within the body cavity should not serve as storage places for spermatozoa. He observed that when ampullae were separated from the rest of the tract, spermatozoa contained therein survived for a short time only. However, when the epididymis was isolated *(7)*, spermatozoa in it

survived for a long time. Kirillov *(6)* further suggested that if the ejaculate was composed of ampullar spermatozoa, and if the bull was not used every day, then ejaculates collected at longer intervals would be of poor quality.

Kirillov *(6)* visualized a regular passage of sperm from the cauda into the ampulla under the influence of sexual stimulation. Cross *(3)* thinks that in the male oxytocin is released during coitus and induces movements of epididymal tubules. Gunn *(5)* and Marshall and Halnan *(10)* have observed epididymal movements during the ejaculatory process, but whether oxytocin is the causative factor is not known. Results of this study, as well as observations of the preceding authors, support the contention of Young *(18)* that the epididymis is the storehouse for spermatozoa.

Phase II. In the cauda epididymis there were on the average $5{,}889.8 \times 10^6$ spermatozoa, in the corpus $2{,}377.6 \times 10^6$, and in the caput $4{,}699.9 \times 10^6$. In terms of percentages the distributions were as follows: cauda—45.42%; corpus—18.34%; caput—36.24%. Comparison of the values for cauda epididymis that were obtained during this phase, with the ones obtained in Phase I, shows that the values obtained by the maceration technique were about three billions lower. The flushing-out technique is considerably more tedious, but seemingly gives more accurate results from the standpoint of actual sperm contents.

The above values were obtained by counting epididymides from 46 mature dairy bulls and were obtained during an 8-mo. interval in 1956. Only during the months of June and July did the caput contain a somewhat larger number of sperm than the cauda. The possible significance of this observation is not fully understood, and it is complicated by the variation in sperm numbers among bulls within individual months.

Phase III. Table 2 contains the between-bull correlation coefficients for the various characteristics studied. The coefficients were calculated by the analysis of covariance *(14)*. The correlations obtained were not significant.

The data of Ortavant *(11)* for the ram, and those of Willett and Ohms *(17)* for the bull, indicate that a significant correlation exists between the testicular size and the sperm production. In electro-ejaculated rams Ortavant obtained high correlations between the testicular and epididymal sperm numbers. Data of this nature are not available for the bull.

A possible explanation that can be advanced for the low correlation coefficients obtained is that the bulls used in our study represented a number of age groups. In a series of exhaustion studies, Willett and Ohms *(17)* obtained for young bulls a correlation of .91 and for old bulls .53, between the scrotal circumference and average sperm number output. If their data are representative of the bull population, then pooling of their data would tend to lower their correlations and possibly bring them into the range observed in this study. The relatively small sample number in our study may account for the statistical nonsignificance of the correlation coefficients obtained. It is of interest that the correlation between weight of the cauda epididymis and the number of sperm in the cauda is twice

TABLE 2

Correlations between testicular and epididymal weights and the number of spermatozoa in the cauda and the caput[a]

Variables	Bulls No.	Correlation coefficient
Testicle weight and sperm number in the cauda	12	0.16
Whole epididymis weight and sperm number in the cauda	12	0.32
Cauda weight and sperm number in the cauda	12	0.37
Caput weight and sperm number in the cauda	6	0.36
Testicle weight and sperm number in the caput	6	0.20
Whole epididymis weight and sperm number in the caput	6	0.27
Caput weight and sperm number in the caput	6	0.34

[a] The correlation coefficients are on a "between-bull" basis.

as large as that between testicular weight and the cauda sperm number. The weight of the cauda might express the storage capacity and would not be expected to diminish with age; whereas, the capacity of the testicle to produce spermatozoa does diminish with age after a maximum has been reached.

The number of spermatozoa in cauda epididymis depends both on the rate of sperm production and on the rate of sperm elimination, so that the caudal contents could vary considerably, depending on the previous sexual activity. The sperm number in the caput epididymis depends on the spermatogenic rate and should reflect the testicular sperm output more closely than does the caudal sperm content. The correlation coefficient between testicular weight and the sperm number in the caput is somewhat higher than that between testes weight and caudal sperm numbers. In the case of the caput, the presence of bulls of various age groups may also account for the low correlations. It would be of interest to conduct a study of this nature with a bull population of known age and of known sexual activity.

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METABOLISM OF BOVINE SEMEN. IV. EFFECTS OF GLYCINE AND GLYCEROL ON THE GLYCOLYTIC AND RESPIRATORY ACTIVITY OF BOVINE SPERMATOZOA[1]

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SUMMARY

Comparisons of the glycolytic activity of bovine spermatozoa at 37° C. over a 3-hr. period have been made in heated milk (M), milk-glycine (MG), milk-glycerol (M-glycerol), and milk-glycine-glycerol (MGG), and in Ringer-phosphate (R), Ringer-phosphate-glycine (RG), and Ringer-phosphate-glycine-glycerol (RGG). Measurements of lactate revealed greater accumulation in M and in R than in the glycine and/or glycerol-containing diluents. Fructose changes were variable and showed no consistent trends.

When the oxygen uptake by washed spermatozoa was measured in the milk diluents, only slightly higher values were obtained in MG and MGG than in M, but when saline was used instead of milk, appreciable stimulation of oxygen uptake occurred in the glycine and/or glycerol-containing preparations.

In the search for improved diluents for bovine semen, the amino acid glycine has received consideration from time to time *(1, 5, 8, 9, 13, 17, 18)*. It has been noted that glycine improved both the survival and fertility of spermatozoa of lower animals *(15, 16)*, but it was not until 1954 that Roy and Bishop *(13)* successfully used glycine in combination with egg yolk as a diluent for laboratory storage of bovine semen. More recently, we have reported *(5)* that combinations of milk and glycine or milk, glycine, and glycerol supported spermatozoan livability at 5° C. for several days longer than did milk without additives.

A knowledge of the mechanism involved in this improved survival of spermatozoa would be of value in attempts to improve semen diluents further. The present report involves studies of the effects of glycine and glycerol on the glycolytic and respiratory activities of spermatozoa in milk diluents, in Ringer-phosphate, and in saline.

EXPERIMENTAL PROCEDURE

The diluents used in these trials were prepared as follows: pasteurized whole milk was centrifuged in the laboratory and the skimmilk (M) obtained was used after heating at 92° C. for 10 min. *(14)*; (MG) one volume of heated skimmilk was mixed with one volume of 0.5 *M* aqueous glycine; (MGG) glycerol was added to MG to a final concentration of 5% glycerol; (M-glycerol) glycerol was added to heated milk to 10% concentration; (R, RG, and RGG) Ringer-phosphate replaced milk in M, MG, and MGG, respectively; (S, SG, and SGG) buffered saline replaced milk in M, MG, and MGG, respectively.

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Fructose utilization and lactate accumulation in each diluent were determined on individual ejaculates. After semen samples were collected with the artificial vagina and subjected to routine laboratory evaluation tests, individual ejaculates were diluted in each diluent included in the trial to a concentration of 50×10^6 spermatozoa per milliliter. Fifteen-milliliter samples were incubated in 15- by 125-mm. test tubes for 3 hr. at 37° C.

Aliquots for chemical analysis were removed after 0 and 3 hr. of incubation and dispersed immediately in 10% trichloroacetic acid. Filtrates subsequently were analyzed for fructose *(12)* and lactate *(2)*.

Measurements of oxygen uptake were made with pooled ejaculates from three to five bulls. After mixing the ejaculates, spermatozoa were washed twice and suspended in the diluents being tested. Spermatozoan concentration was standardized at 250×10^6 cells per milliliter and fructose concentration at 0.01 *M* in the oxygen uptake trials.

RESULTS AND DISCUSSION

Experiment 1 involved a comparison of fructose disappearance and lactate accumulation in M, MG, and MGG, and the results are summarized (Table 1).

TABLE 1

Fructolysis in milk diluents

	Diluent			
	M	MG	MGG	M-Glycerol
	Experiment 1 (mean of 29 ejaculates)			
Fructose loss[a]	1.09 ± 0.13[b]	1.52 ± 0.20	1.33 ± 0.21	
Lactate gain[a]	1.49 ± 0.16	0.93 ± 0.11	0.82 ± 0.10	
Ratio $\frac{\text{lactate gain}}{\text{fructose loss}}$	1.37	0.61	0.62	
	Experiment 3 (mean of 20 ejaculates)			
Fructose loss	1.18 ± 0.13	1.12 ± 0.10	1.10 ± 0.11	0.68 ± 0.14
Lactate gain	2.06 ± 0.08	1.58 ± 0.10	1.17 ± 0.07	1.33 ± 0.07
Ratio $\frac{\text{lactate gain}}{\text{fructose loss}}$	1.75	1.41	1.06	1.95

[a] Milligrams per hour per 10^9 spermatozoa.
[b] Standard error of the mean.

Significantly less lactate accumulated in MG and MGG than in M ($P = 0.01$). There appeared to be more fructose utilization in MG and MGG than in M, although the results on individual ejaculates in MG and MGG varied considerably.

The reduced accumulation of lactate in MG and MGG may have been due to specific effects of glycine and glycine-glycerol, or it may have been due to interactions between these substances and milk. In an attempt to resolve this point, a second experiment was conducted in which Ringer-phosphate was substituted for milk in each of the diluents used in Experiment 1. The results of Experiment 2 are summarized in Table 2; as in Experiment 1, lactate accumulation

TABLE 2

Fructolysis in Ringer-phosphate diluents
(Mean of 11 ejaculates)

	Diluent		
	R	RG	RGG
Fructose loss[a]	0.88 ± 0.13[b]	0.60 ± 0.13	0.58 ± 0.11
Lactate gain[a]	1.67 ± 0.12	1.10 ± 0.09	1.02 ± 0.08
Ratio $\frac{\text{lactate gain}}{\text{fructose loss}}$	1.90	1.83	1.76

[a] Milligrams per hour per 10^9 spermatozoa.
[b] Standard error of the mean.

was reduced 35–40% in the presence of glycine or glycine-glycerol. Fructose disappearance appeared to be slightly less in R (Table 2) than in M (Table 1), but in RG and RGG less fructose disappeared than in R, in contrast to the apparent increased fructose disappearance noted when additions of glycine and glycine-glycerol were made to M.

Experiment 3 was conducted using M, MG, and MGG as employed in Experiment 1, plus a fourth diluent, M-glycerol. As shown in Table 1, lactate accumulation was 23, 35, and 43% less in MG, M-glycerol, and MGG than in M. Fructose disappearance may have been slightly reduced in MG and MGG, and was considerably reduced in M-glycerol. Three of the 20 M-glycerol samples actually showed an accumulation of fructose for the 3-hr. period. This is in accord with the reported fructose-sparing action of glycerol *(10, 11)*.

The variable results obtained in these studies with regard to fructose utilization are not easily explainable. Past experience in this laboratory has been that fructose determinations are less likely to vary unaccountably than lactate determinations. While the Roe method is reasonably specific for fructose, it is possible that some sort of interaction in MG and MGG interfered with the fructose assays. The results in Ringer-phosphate (Table 2, reduced disappearance of fructose in RG and RGG) are in agreement with isotopic studies in which $C^{14}O_2$ production from glucose-C^{14} was reduced when glycine was added to the system *(4)*.

These three experiments involving 60 ejaculates show that lactate accumulation is reduced in the presence of glycine and/or glycerol, under the conditions employed. On the other hand, no conclusions can be drawn regarding the effects on fructose disappearance. Reduced lactate accumulation in the absence of reduced fructose disappearance might be accounted for by conditions favoring oxidation of the lactate formed. To test this possibility, oxygen uptake was measured in M, MG, MGG, S, SG, and SGG. Buffered saline was used instead of Ringer-phosphate to avoid the depressing effect of phosphate on oxygen uptake *(3, 10)*. As shown in Table 3, oxygen uptake in the saline-containing diluents was stimulated by glycine and glycine-glycerol additions. In milk, the increase in oxygen uptake produced by these additions was much less. However, the 4 to

10% increase in oxygen uptake (Table 3) may account for part of the 23–45% reduction in accumulated lactate (Table 1) in MG and MGG than in M.

TABLE 3

Oxygen uptake by washed spermatozoa
(Mean of four trials)

	None	Glycine	Glycine plus glycerol
	$-Zo_2$[a]	$-Zo_2$	$-Zo_2$
Saline	7.1 ± 2.3	11.5 ± 1.9	10.1 ± 1.6
Milk	9.9 ± 2.0	10.9 ± 2.0	10.3 ± 1.7

[a] Micro liters O_2 per 10^9 cells per hour.

The increase in oxygen uptake in SG and SGG is believed not due to oxidation of glycine. While $C^{14}O_2$ is recovered in good yield when spermatozoa are incubated with glycine-C^{14} *(4, 7)*, manometric studies have failed to demonstrate oxygen uptake above endogenous levels with glycine as the substrate *(7)*. Lactate could be removed without oxygen uptake by conversion to pyruvate and subsequent transamination. There is evidence of such transamination reactions in bovine spermatozoa *(6)*, but their quantitative importance under the conditions involved in the present study has not been established.

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FREEZING BOVINE SEMEN. IV. EFFECT OF FREEZING ON THE METABOLIC ACTIVITY OF BOVINE SPERMATOZOA DURING AND AFTER STORAGE AT −79° C.[1, 2]

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SUMMARY

No evidence of respiratory activity by bull spermatozoa diluted in skimmilk-glycerol during storage for 6 mo. at −79° C. could be demonstrated as measured by production of $C^{14}O_2$ from radioactive substrates present in the frozen medium. However, freezing resulted in harmful effects on post-thawing metabolism of spermatozoa. After 6 mo. at −79° C., twice-washed spermatozoa showed 34% less $C^{14}O_2$ production from glucose-U-C^{14} during post-thawing incubation for 6 hr. at 5° C. than during a similar prefreezing incubation period. Unwashed sperm, frozen and stored for 1 hr., produced 61% less lactic acid during post-thawing incubation for 3 hr. at 37° C. than sperm from the same semen samples which were not frozen. The 36% loss of motile sperm due to freezing was not sufficient to explain the greatly reduced lactic acid production. Sperm stored frozen for 1 hr. in skimmilk-glycerol showed significantly greater post-thawing lactic acid production during incubation under air than those frozen in 1:4 egg yolk–citrate-glycerol, even though fewer sperm survived freezing in skimmilk. Adding 1.25% fructose to skimmilk-glycerol and egg yolk–citrate-glycerol diluents resulted in greater lactic acid production by both frozen, thawed and unfrozen spermatozoa. However, frozen samples still showed greatly depressed metabolic activity as compared to unfrozen samples.

The effect of freezing on the metabolic activity of bull spermatozoa has received little attention. It is assumed that metabolic activity ceases during storage at −79° C. or below, and that spermatozoa are in a state of suspended animation *(21)*. No studies have been conducted to determine whether spermatozoa exhibit metabolic activity at these low temperatures.

Freezing is harmful to the post-thawing metabolism of various tissue preparations *(7, 9, 22)*; whereas, human red blood cells appear to survive freezing and storage at −79° C. with very little if any alteration of metabolic activity *(3)*. However, as pointed out by Lovelock and Polge *(14)*, spermatozoa of the rabbit, bull, and fowl are more sensitive to freezing damage than human red blood cells. Also, it has been observed by several workers *(18, 19, 25, 27)* that bull spermatozoa show a more rapid loss of motility during post-thawing storage at 5° C. than during storage at 5° C. as unfrozen semen. O'Dell and Almquist *(19)* noted that spermatozoa frozen to −79° C. in milk diluents retained higher

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motility over a 48-hr. period at 5° C. after thawing than did those frozen in egg yolk–citrate. However, immediately after freezing, motility in the two diluents was essentially equal. This observation was confirmed by O'Dell and Hurst *(18)*.

Using egg yolk diluents, respiration *(10, 25, 27)* and glycolysis *(27)* of frozen and thawed semen were found to be greatly depressed and decreased more rapidly with time than did those of the unfrozen controls.

This paper reports the results of experiments designed to determine: *(a)* if metabolic activity of bovine spermatozoa could be detected during storage at −79° C., and *(b)* the effect of heated skimmilk and egg yolk–citrate diluents on post-thawing lactic acid production by bovine spermatozoa after freezing to −79° C.

EXPERIMENTAL PROCEDURE

Experiment 1. Metabolic activity of spermatozoa during storage at −79° C. Two trials were conducted. In the first trial, 3.5×10^9 unwashed spermatozoa from pooled ejaculates were diluted with 2.5 ml. of heated skimmilk diluent and cooled to 5° C. A total of five tubes was prepared. After cooling, 2.5 ml. of skimmilk–20% glycerol were added in three equal portions at 10-min. intervals to each of the five tubes of diluted semen. Ten μc. of glycerol-1-C^{14} were added to each tube prior to the addition of the glycerol-skimmilk solution. One thousand units each of penicillin and streptomycin per milliliter of diluted semen were used. The spermatozoa in two of the tubes were inactivated with one drop of 10 *N* sulfuric acid, to serve as controls. The tubes were flushed with nitrogen, sealed, and allowed a 6-hr. glycerol equilibration period at 5° C. prior to freezing. The CO_2 produced by the spermatozoa during equilibration was recovered and assayed for radioactivity according to procedures previously described *(6, 20)*. The semen samples were transferred to a 5° C. ethyl alcohol bath in a 1-gal. Dewar vacuum flask and were frozen by the gradual addition of dry ice to lower the temperature of the alcohol bath at the rate of 3° C. per minute from 5 to −15° C., and 10° C. per minute from −15 to −79° C. The tubes then were flushed with nitrogen and sealed for storage at −79° C. for 1 mo.

After 1 mo. of storage, the CO_2 in the tubes was flushed with nitrogen into 10 ml. of 1 *N* NaOH, converted to $BaCO_3$, and assayed for radioactivity. After recovery of the CO_2, the tubes of frozen semen were thawed in a 5° C. water bath and the percentage of spermatozoa surviving 1 mo. of storage at −79° C. was estimated.

In the second trial, both whole semen and twice-washed spermatozoa (with 0.9% saline) were diluted with heated skimmilk and frozen. Five billion sperm per tube were used and 5 μc. of uniformly labeled glucose (glucose-U-C^{14}) replaced glycerol-1-C^{14} as the labeled substrate. The frozen semen samples were stored for 6 mo. at −79° C. However, the CO_2 present in the tubes of frozen semen was recovered after both 1 and 6 mo. of storage. The remainder of the procedure was the same as that described for the first trial, except that after thawing, the semen samples were flushed with nitrogen, sealed, and stored at

5° C. for 6 hr. to provide information on the effect of freezing and storage on the post-thawing metabolic activity of the spermatozoa.

Experiment 2. Effect of diluent on post-thawing lactic acid production by bull spermatozoa. This experiment was designed to compare the effect of heated skimmilk and 1 : 4 egg yolk–citrate diluents containing 10% glycerol by volume on post-thawing lactic acid production by unwashed spermatozoa. Three replicates were conducted.

Each diluent was tested with and without the addition of 1.25% fructose by weight. The method of processing the semen prior to freezing was the same as that described in Experiment 1. However, in the present experiment, the volume of pooled semen required to furnish 250×10^6 motile spermatozoa per milliliter of diluted semen was taken into consideration for providing a final concentration of 10% glycerol in the medium. The fructose was added to the diluents by dissolving twice the desired final concentration of 1.25% in the glycerol fraction, as recommended by Hafs and Elliott *(8)*.

Four tubes of diluted semen were prepared for each of the four diluent combinations. Immediately after addition of the glycerol fractions, two tubes were transferred to a 37° C. water bath while the other two were placed in a 5° C. ethyl alcohol bath and frozen to −79° C. by the same procedure used in Experiment 1. After 1 hr., the tubes of frozen semen were thawed in water at 37° C. and transferred to the incubation water bath. Both the frozen and unfrozen semen samples were incubated under air for 3 hr. at 37° C. Spermatozoan motility was estimated at the beginning and end of the incubation period.

One tenth-milliliter portions of diluted semen were removed at the beginning and end of the incubation period and deproteinized by adding 4.9 ml. of cold 10% trichloroacetic acid. This mixture was allowed to stand for 20 min. and then filtered. Lactic acid in the filtrates was determined by the method of Barker and Summerson *(2)*. Colorimetric determinations were made in duplicate and averaged, for reporting lactic acid values. These values were calculated as milligrams of lactic acid produced per 10^9 spermatozoa per hour at 37° C.

RESULTS AND DISCUSSION

Experiment 1. As shown (Tables 1 and 2), assay of the CO_2 recovered after 1 mo. of storage at −79° C. revealed no radioactivity in any of the tubes of frozen semen containing live spermatozoa, irrespective of whether labeled glycerol or glucose was present. Control samples containing inactivated spermatozoa also showed no radioactivity.

Although it appears that a slight amount of radioactive CO_2 was present after 6 mo. of storage (Table 2), it can be seen that the control samples containing inactivated sperm also exhibited approximately the same amount of radioactivity. Therefore, under the conditions of this experiment, bull spermatozoa did not exhibit any respiratory activity during storage at −79° C. for 6 mo., as measured by the production of $C^{14}O_2$ from labeled substrates present in the frozen medium.

TABLE 1

Metabolism of glycerol-1-C^{14} by unwashed spermatozoa in skimmilk–10% glycerol during storage at 5 and −79° C.

Tube	Specific activity of $C^{14}O_2$[a] after: 6 hr. at 5° C. before freezing	1 mo. at −79° C.	% motile sperm after: 6 hr. at 5° C. before freezing	1 mo. at −79° C.
		Unwashed spermatozoa		
1	13	0	50	20
2	16	0	45	20
3	15	0	50	20
Av.	15	0	48	20
		Control—inactivated spermatozoa		
1	6	0	0	0
2	0	0	0	0
Av.	3	0	0	0

[a] Counts per minute per milligram of $BaCO_3$.

TABLE 2

Metabolism of glucose-U-C^{14} by washed and unwashed spermatozoa diluted in skimmilk–10% glycerol during storage at 5 and −79° C.

Tube	Specific activity of $C^{14}O_2$[a] after: 6 hr. at 5° C. before freezing	1 mo. at −79° C.	6 mo. at −79° C.	6 hr. at 5° C. post-thawing	% motile sperm after: 6 hr. at 5° C. before freezing	6 mo. at −79° C.
			Unwashed spermatozoa			
1	25	0	5	25	50	15
2	26	0	3	27	40	15
3	26	0	2	23	50	20
Av.	26	0	3	25	47	17
			Washed spermatozoa			
1	134	0	3	93	50	20
2	128	0	2	89	50	20
3	145	0	2	88	50	20
Av.	136	0	2	90[b]	50	20
			Control—inactivated unwashed spermatozoa			
1	0	0	4	2	0	0
2	0	0	7	2	0	0
Av.	0	0	5	2	0	0

[a] Counts per minute per milligram of $BaCO_3$.
[b] Significantly lower than the average of 136 counts per minute observed during storage for 6 hr. at 5° C. before freezing.

However, longer periods of storage and development of other techniques for measurement of spermatozoan metabolism at temperatures of −79° C. or below might reveal a very low rate of metabolic activity taking place at these temperatures. With chironomid larvae, respiratory exchanges have been observed at temperatures as low as −38° C. *(23)*. However, with present techniques for freezing and storage, bull spermatozoa will not survive for prolonged periods of time at temperatures above −70° C. *(16)*.

Although certain enzymes have been found to be active at temperatures

ranging from −15 to −30° C. *(1, 24)*, the velocity of enzyme action was considerably reduced *(11)*. Sizer and Josephson *(24)* found that during several weeks at −70° C. no hydrolysis by lipase, trypsin, and invertase preparations could be detected. As pointed out by White *(26)*, it is very unlikely that the protective action of glycerol during freezing and storage is dependent on its ability to support metabolism of spermatozoa at these temperatures. The relatively high concentrations (7 to 13% by volume) necessary for obtaining optimum survival of sperm after freezing, point toward the importance of the biophysical effects *(13, 14)* of glycerol.

As shown (Table 2), freezing and storage for 6 mo. at −79° C. had no apparent effect on the post-thawing respiratory activity of unwashed sperm as measured by the difference in radioactivity of the CO_2 produced during post-thawing and prefreezing incubation intervals of 6 hr. at 5° C. However, with twice-washed sperm, post-thawing production of $C^{14}O_2$ from labeled glucose was only 66% of that observed during the prefreezing storage interval. This difference was significant at the 5% level of probability as determined by the "t" test. Approximately the same percentage of washed and unwashed sperm survived 6 mo. of storage at −79° C. Therefore, the washing procedure may have rendered the sperm cells more susceptible to freezing injury, with the result that twice-washed sperm showed a greater reduction in post-thawing respiratory activity than did unwashed sperm.

Experiment 2. Table 3 shows the effect of skimmilk and 1 : 4 egg yolk–citrate diluents containing 10% glycerol by volume on post-thawing lactic acid production by unwashed bull spermatozoa after freezing to −79° C. Irrespective of diluents, the average lactic acid production by frozen and thawed sperm was only

TABLE 3

Effect of freezing on lactic acid production and motility of spermatozoa in heated skimmilk and 1 : 4 egg yolk–citrate diluents containing glycerol and fructose

(Average of three replicates)

Diluent	Lactic acid production[a] by		Ratio $\frac{\text{Frozen}}{\text{Unfrozen}}$	% motile sperm		
	Unfrozen sperm	Frozen, thawed sperm		Before freezing	After freezing	After post-thawing incubation
Skimmilk-glycerol						
Without fructose	1.20	0.51	0.42	47	28	16
With fructose	1.45	0.64	0.44	48	28	19
Av.	1.33	0.58	0.43	48	28	18
Egg yolk–glycerol						
Without fructose	1.48	0.51	0.34	52	35	15
With fructose	1.89	0.71	0.38	54	38	23
Av.	1.68	0.61	0.36	53	36	19
Combined						
Without fructose	1.34	0.51	0.38	49	31	16
With fructose	1.67	0.67	0.40	51	33	21
Av.	1.50	0.59	0.39	50	32	18

[a] Milligrams of lactic acid produced per 10^9 spermatozoa per hour at 37° C.

39% of that observed for the unfrozen controls. This difference was highly significant ($P < 0.01$). The loss of motile sperm due to freezing was only 36% ($P < 0.05$), so that the reduced post-thawing lactic acid production can not be attributed entirely to the loss of motile sperm during freezing. Apparently, there was some impairment of the metabolic activity of the spermatozoa which survived freezing.

White *et al. (27)* reported somewhat similar results based on oxygen consumption by bull spermatozoa in egg yolk–phosphate-fructose diluent before and after freezing. Frozen and thawed sperm showed a 76% reduction in oxygen uptake, but only a 53% loss of motile sperm due to freezing. In the present study, the sperm were stored at −79° C. for only 1 hr. Other studies with frozen kidney mitochondria *(12)* and rabbit brain, liver, muscle, and skin preparations *(9)* have shown that freezing not only damages post-thawing metabolic activity but that the damage increases with exposure time. Additional studies of this phenomenon with bull sperm would be of interest, even though it appears that fertility of frozen bull sperm does not decrease greatly with time *(15, 17)*.

Lactic acid accumulation was slightly greater in the presence of fructose and glycerol than when only glycerol was present. Although this may be simply the result of the presence of a larger amount of more readily available substrate, it may aid in explaining the improved survival of sperm after freezing in diluents with added sugars *(4, 5, 19)*. Regardless of the effect of fructose in increasing lactic acid production, frozen and thawed samples still showed greatly depressed metabolic activity as compared to unfrozen samples.

Analysis of variance showed a highly significant interaction ($P < 0.01$) between diluents and the effect of freezing on lactic acid production. Whether fructose was present or not, the depression in lactic acid production due to freezing was greater in semen samples frozen in egg yolk–citrate than in those frozen in skimmilk diluent (64 and 57%, respectively). This interaction appears even more prominent in view of the fact that only 28% motile sperm were present in skimmilk after freezing, while 36% were present in yolk-citrate diluent. These results indicate that sperm frozen in skimmilk diluent were more active metabolically during post-thawing storage than those frozen in yolk-citrate diluent. This offers a possible explanation for the ability of bull spermatozoa to maintain higher motility during post-thawing storage in milk than in yolk-citrate diluent, as observed both in the present study and in a previous study *(19)* in this laboratory. In the present study, frozen and thawed sperm showed highly significant declines of 10 and 17 percentage units of motility during 3 hr. at 37° C. in skimmilk and yolk-citrate diluents, respectively. Unfrozen sperm showed no significant change in motility during the incubation period.

The severe reduction in motility and metabolic activity of spermatozoa after freezing and thawing provides additional support for the common practice of using frozen semen for breeding purposes as soon as possible after thawing. However, the present study indicates that this may not be quite as critical with semen frozen in milk diluents. Additional research is planned to determine the

specific action(s) involved in the greater ability of milk than of egg yolk–citrate to reduce the harmful effects of freezing on the metabolic activity of bull spermatozoa.

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FERTILITY OF BULL SPERMATOZOA IN YOLK-CITRATE PARTIALLY REPLACED BY GLUCOSE

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SUMMARY

Four livability trials and three breeding trials were conducted to compare two diluters: *(a)* YGC—one part egg yolk, four parts of a 5% solution of glucose, and one part of a 2.9% solution of sodium citrate dihydrate, and *(b)* YC—one part egg yolk and one part of a 2.9% solution of sodium citrate dihydrate. The means of the progressive motilities for 50 ejaculates stored in these diluters for seven days at 5° C. were not significantly different ($P > 0.05$). After one day of storage, the difference between the average nonreturn rates for the two diluters was not significant ($P > 0.05$). There was a total of 6,405 first services. After two days of storage, with a total of 1,914 services, the 60- to 90-day nonreturn rate with YGC was 61.7, and with YC, 55.7. The difference is highly significant ($P < 0.01$). These data support published livability studies which have indicated that a high concentration of electrolytes is detrimental to spermatozoa.

Kampschmidt *et al. (1, 2)* demonstrated that high concentrations of electrolytes were detrimental to spermatozoa during storage and that the replacement of most of the electrolytes by glucose, a sugar metabolizable by spermatozoa, was beneficial. Livability studies in the present authors' laboratory indicated that replacement of two-thirds of yolk-citrate with glucose might be beneficial. Breeding trials were, therefore, conducted to compare yolk-citrate with yolk-citrate-glucose. This paper reports the results.

EXPERIMENTAL PROCEDURE

In four separate livability trials, the two diluters to be compared were as follows: *(a)* One part egg yolk, four parts of a 5% solution of glucose, and one part of a 2.9% solution of sodium citrate dihydrate. This diluter was one studied by Kampschmidt *et al. (2)*. *(b)* One part egg yolk and one part of a 2.9% solution of sodium citrate dihydrate. Dihydrostreptomycin sulfate was added to both diluters at the rate of 500 γ per milliliter. In each, semen was diluted to contain 15×10^6 spermatozoa per milliliter. After storage at 5° C. for seven days, motility estimations were made on each sample independently by two workers and the two values were averaged.

A comparison of the two diluters was made in three separate breeding trials. The yolk-citrate diluent was routinely employed in the bull stud in which these tests were conducted. In the first trial, 500 γ of dihydrostreptomycin sulfate were added per milliliter of diluent. In the second and third trials, dihydrostreptomycin sulfate at the rate 500 γ per milliliter and sulfanilamide at the rate of 0.3% were added. Dilution rates varied from 1:76 to 1:338, and the number of

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spermatozoa in each milliliter of diluted semen varied from 9 to 34×10^6. Due to the limited number of services, the collections were not split *(4)*. In the first trial, there were two collections from each of eight bulls; in the second, two collections from each of 19 bulls, and in the third, six or eight collections from each of eight bulls, with a total of 44 collections. In every instance, the treatments within each bull were alternated in the successive weekly collections. In each week of each experiment, the semen from one-half of the bulls received one treatment and the semen from the other half of the bulls received the other treatment, to minimize time effects. Breeding efficiency was measured by nonreturn rates determined from 60 to 90 days after first services.

RESULTS AND DISCUSSION

The livability data are summarized (Table 1). The difference between the means is not significant ($P > 0.05$), as established by analysis of variance. These results in conjunction with the pH determinations indicate that the reduced buffer concentration in the yolk-glucose-citrate was still adequate to maintain optimum pH with only 15×10^6 spermatozoa per milliliter. Kampschmidt *et al. (1, 2)* reported difficulty in maintaining optimum pH in the yolk-glucose-citrate diluter when the semen was diluted 1:10. Also, with the low concentration of sperm employed in the present experiments, and in light of the study of glycolysis by spermatozoa in yolk-citrate and yolk-glucose-citrate by Salisbury and VanDemark *(3)*, it is doubtful if metabolizable sugar could have been a limiting factor in the yolk-citrate in the present study, although those workers did observe some stimulation of spermatozoa with addition of glucose.

The number of services and the nonreturn rates for the three breeding trials and for all trials combined are shown (Table 2). When the data from all three trials are combined, the difference between the means is not significant ($P > 0.05$), as established by analysis of variance, for services performed the first day after collection. For semen stored two days, the over-all average nonreturn rate of spermatozoa in yolk-glucose-citrate is highly significantly greater ($P < 0.01$)

TABLE 1

Progressive motility of spermatozoa and pH of semen after storage in yolk-citrate and yolk-glucose-citrate for seven days at 5° C.

			Yolk-citrate		Yolk-glucose-citrate		
			pH			pH	
Trial	Ejaculates	Motility	Initial	After storage	Motility	Initial	After storage
(No.)	*(No.)*	*(%)*			*(%)*		
17	10	31.5	6.84	6.74	29.3	6.94	6.78
18	13	23.8	6.81	6.63	33.2	6.96	6.69
20	12	43.5	.....	.....	45.0	.....	.....
23	15	34.5	.....	.....	31.5	.....	.....
Totals and averages	50	33.3	.....	.....	34.7	.....	.....

TABLE 2

Sixty- to ninety-day nonreturn rates with spermatozoa stored at 5° C. for one and two days in yolk-citrate and in yolk-citrate-glucose

	Yolk-citrate		Yolk-citrate-glucose	
	Services	Nonreturns	Services	Nonreturns
	(No.)	*(%)*	*(No.)*	*(%)*
One-day storage				
Experiment 1	365	76.7	405	71.6
Experiment 2	1,678	67.5	1,631	72.5
Experiment 3	1,196	60.5	1,130	59.8
Totals and averages	3,239	66.0	3,166	67.8
Two-day storage				
Experiment 1	318	65.7	330	64.5
Experiment 2	344	53.5	344	68.6
Experiment 3	297	47.5	281	49.8
Totals and averages	959	55.7	955	61.7

than that in yolk-citrate. These data were analyzed by Chi-square, because of the small number of services in most of the collections. The results indicate that yolk-citrate as a diluter for bull spermatozoa is improved by replacement of two-thirds of it by glucose. These breeding results support the observations made by Kampschmidt *et al. (1, 2)*, that a high concentration of electrolytes is detrimental to spermatozoa.

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EFFECT OF PH ON THE LIFE-SPAN AND METABOLISM OF BOVINE SPERM KEPT AT ROOM TEMPERATURES [1, 2]

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SUMMARY

The oxygen consumption, lactic acid production, motility, pH, and survival rate of bovine spermatozoa suspended in coconut milk diluent: *(1)* CMC, consisting of a 1:1 mixture of boiled coconut milk and a 4.32% solution of sodium citrate, and *(2)* CMCC, consisting of CMC, which contained 100 mg. % calcium carbonate, were measured after 3, 76, and 150 hr. of storage at room temperatures. With the progression of time, lactic acid accumulated, causing a gradual decline in metabolic activity which was significantly correlated with the increased hydrogen ion concentration. In a pH range of 5.50–5.80, the sperm appeared to be maximally inhibited, but sustained a comparatively insignificant loss of viability. Resuspending the auto-inhibited spermatozoa in fresh, relatively alkaline coconut milk diluents produced an appreciable resumption of metabolic activity after six days of room temperature storage. The evidence strongly suggests that reversible acid inhibition can serve as a means of extending the life-span of sperm for several days at room temperatures.

Although reversible inhibition of spermatozoal biochemical activity by antimetabolites *(2, 12, 13, 14, 18)*, increased hydrogen ion concentration *(5, 7, 9, 19)*, and narcotizing agents such as alcohol, ether, nitrous oxide, and carbon dioxide *(21, 22)* has been described in the literature, relatively few attempts have been made to utilize reversible chemical inhibition as a means of prolonging the life-span of spermatozoa at room temperatures.

In 1927, Kryzsyzkowsky and Pawlow *(10)* noted that sperm imprisoned in sealed tubules at room temperature became immobile and that subsequent exposure to air produced a resumption of activity. They postulated that accumulated carbon dioxide narcotized the sealed-in sperm and that when the carbon dioxide was permitted to escape sperm motility was restored. Shettles *(22)* found that carbon dioxide reversibly inhibited sperm motility for a short time, while longer exposure of sperm to this gas proved toxic. In a recent article, VanDemark and Sharma *(25)* reported that they were able to maintain bovine sperm under carbon dioxide narcotization for from six to seven days at prevailing room temperatures. According to their results, the sperm showed no loss of fertilizing capacity when they were reactivated.

Lardy and Phillips *(13)* noted that sperm suspended in an egg yolk–citrate

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solution could be inhibited for several days by fluoride, and partially reactivated by washing and resuspending the sperm in fresh diluent. Willett and Ohms *(26)* have recently investigated the reversible inhibitory effect of lactic acid on sperm motility. While the latter two experiments were conducted at low temperatures, they suggest the possible usefulness of these techniques in prolonging sperm viability at room temperatures.

The purpose of the present experiment is to provide evidence that reversible acid inhibition can extend the life-span of an economically usable number of bovine sperm stored at room temperatures in a suitable medium.

EXPERIMENTAL PROCEDURE

General procedure. Pooled ejaculates of freshly collected bovine semen were divided into three equal portions and suspended in the following media: *(1)* Coconut milk—sodium citrate (CMC), consisting of a 1:1 mixture of decanted coconut milk which had been boiled for 10 min. and a 4.32% solution of sodium citrate dihydrate, *(2)* Coconut milk–sodium citrate–calcium carbonate (CMCC), consisting of a 1:1 mixture of boiled coconut milk and a 4.32% solution of sodium citrate containing a final concentration of 100 mg. % of anhydrous calcium carbonate, *(3)* Krebs-Ringer phosphate calcium free buffered solution *(24)* containing 600 mg. % fructose (KRBF). All sperm suspensions contained 66 mg. % penicillin, sodium (1,625 units/mg), and 75 mg. % dihydrostreptomycin sulfate (740 μg/mg). The volume in each case was adjusted to give a final concentration of 2×10^8 sperm/ml. Several 12-ml. aliquots of each of the sperm suspensions were poured into sterile test tubes, sealed, and stored at the prevailing room temperatures (23–27° C.). All glassware used in these procedures was autoclaved, as a precautionary measure against further contamination of the sperm preparations by bacteria and fungi. At the end of approximately 3-, 76-, and 150-hr. intervals, test tubes from each of the three groups were removed from their storage racks and their contents used to measure oxygen consumption, lactate production, motility, number of live sperm, and pH. Several test tubes removed after 76 and 150 hr. of storage were subjected to further treatment before measuring sperm activity. In these test tubes, the old diluent was separated and removed from the sperm in exchange for an equivalent volume of fresh diluent.

Procedure for renewing diluent. All test tubes containing 76-hr.-old and 150-hr.-old sperm suspensions were centrifuged at approximately $650 \times$ g. for 10 min. In one test tube from each of the three groups the sperm were gently resuspended in the original, unchanged diluent. These control tubes served to indicate the effect of the aged, unchanged diluent on sperm metabolism and mortality rate. The old diluents in the remaining tubes were carefully removed with an automatic pipetting device and replaced with equal volumes of appropriate fresh extender. The resuspended sperm in these tubes were used to measure the degree of reactivation induced by the fresh media. For a detailed explanation of the individual treatments, and the symbols used to identify these treatments at various time intervals, see Table 1.

TABLE 1

Effect of pH and age on the respiration, anaerobic glycolysis, motility, and survival rate of sperm stored in various diluents at room temperatures (23–27° C.)[a]

Sperm diluents	Sperm age *(hr.)*	ZO_2	$Z_L^{N_2}$	Motility	Live sperm *(% initial)*	pH range
1. Coconut milk–sodium citrate (CMC)	3	14.1	29.9	4	62	6.52–6.60
2. Coconut milk–sodium citrate–calcium carbonate (CMCC)	3	18.4	50.0	5	62	7.10–7.15
3. Krebs-Ringer phosphate buffered fructose (KRBF)	3	18.9	47.4	5	62	6.70–7.08
1a. CMC (no fresh diluent added)	76	7.8	17.7	2	53	5.76–5.81
1b. CMC (old diluent removed and replaced with fresh diluent for the first time)	76	9.9	22.0	3+	53	6.41–6.64
1c. CMC (old diluent removed and replaced with fresh diluent containing $CaCO_3$ for the first time)	76	10.9	31.6	4	58	7.40–7.53
2a. CMCC (no fresh diluent added)	76	9.2	22.0	3	54	6.09–6.14
2b. CMCC (old diluent removed and replaced with fresh diluent for the first time)	76	11.5	40.0	4	60	7.45–7.80
3a. KRBF (no fresh diluent added)[b]	76	0.0	0.0	0	19	5.40–5.44
3b. KRBF (old diluent removed and replaced with fresh diluent for the first time)	76	0.2	0.8	0	19	6.70–6.80
1A. CMC (no fresh diluent added)	150	2.9	8.6	0	44	5.58–5.70
1B. CMC (old diluent removed and replaced with fresh diluent for the first time)	150	5.6	9.6	2	50	6.34–6.39
1C. CMC (old diluent removed and replaced with fresh diluent containing $CaCO_3$ for the first time)	150	6.6	18.2	3	51	7.08–7.38
2A. CMCC (no fresh diluent added)	150	3.9	8.4	0	44	5.71–5.80
2B. CMCC (old diluent removed and replaced with fresh diluent for the first time)	150	8.4	19.5	3	54	7.09–7.45

[a] Each datum is the mean of six replicates.

[b] KRBF sperm suspensions were discarded at the end of 76 hr., since they no longer contained any measurable activity.

Analytical procedures. Sperm density was determined turbidimetrically in a Klett-Summerson colorimeter and by counting in a hemocytometer. The pH was measured with a Beckman Model G pH meter. Motility ratings were based on microscopic examination of sperm samples after they had been warmed at 37° C. for 30 min. These ratings were expressed in terms of an arbitrary scale ranging from 0–5 as follows: Nonmotile (0), vibratory motility (1), poor progressive motility (2), fair progressive motility (3), good progressive motility (4), excellent progressive motility (5). Live-dead ratios were calculated according to the method described by Campbell *et al. (3)*. Nigrosin eosin was used as the stain for differentiating between live and dead spermatozoa.

The oxygen consumption of the spermatozoal suspensions was measured by means of the direct method of Warburg under air at 37° C. *(24)*. The oxygen uptake is expressed in terms of the coefficient ZO_2, which represents the mm.^{3} O_2 consumed/10^8 sperm/hour at 37° C. The lactic acid produced by sperm kept under nitrogen at 37° C. was measured according to the method of Barker and Summerson *(1)*. Micrograms of lactic acid produced were converted to mm.^{3} CO_2, evolved by dividing by 4.02 *(13)*. The CO_2 production is expressed in terms of the coefficient $Z_L^{N_2}$, which represents mm.^{3} CO_2 evolved/10^8 sperm/hour under nitrogen at 37° C. *(11)*. In all cases, corrections were made for the O_2 uptake and CO_2 production of fresh and aged diluent minus sperm. All analyses were made in duplicate or in triplicate. The data for three replicate experiments were used to compute the significance of differences between means by analysis of variance *(23)*.

RESULTS

Inhibition. The data clearly show that sperm suspended for 3 hr. in CMC (No. 1) are metabolically less active in terms of respiration (Figure 1), anaerobic glycolysis (Figure 2), and motility (Table 1) than sperm maintained for a similar period of time in CMCC (No. 2) or in KRBF (No. 3). This difference in activity approaches statistical significance and appears to be a function of pH. The hydrogen ion concentration in Diluent 1 was significantly lower ($P < .05$) than the hydrogen ion concentration in Diluents 2 and 3. The fact that the sperm at this time are relatively fresh, and that the live-dead ratio (Table 1) is the same in all three diluents, provides further evidence for associating the lowered metabolic rate of the sperm suspended in No. 1 with acidity and not with senescence or increased mortality rate.

The sperm which remained suspended in unchanged diluent for more than 3 hr. manifested a progressive decline in activity concomitant with increased age, hydrogen ion concentration (the principal source of hydrogen ion, as determined by chemical analysis, was lactic acid), and increased mortality. Each of these factors can contribute singly or in combination, and in varying degree, towards inhibition of sperm activity. For example, the virtually complete loss of activity of 76-hr.-old sperm (3a) suspended in KRBF is attributable to the high mortality

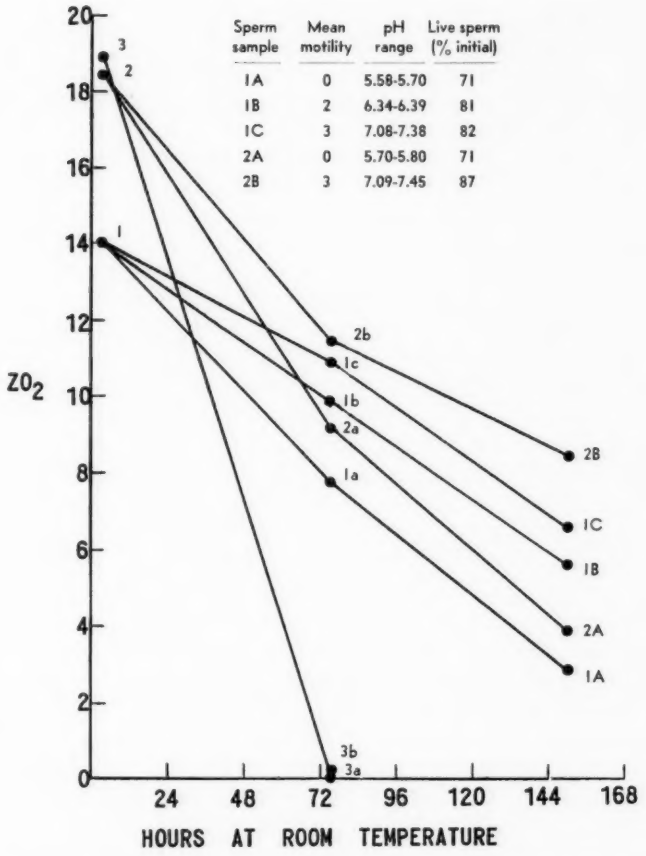

FIG. 1. Oxygen consumption of bovine sperm suspended in *(1)* coconut milk–sodium citrate diluent, *(2)* coconut milk–sodium citrate–calcium carbonate diluent, *(3)* Krebs-Ringers phosphate buffered fructose diluent; (A, a) signifies diluent indicated by number was not renewed (a) after 76 hr. of storage, (A) after 150 hr. of storage, (B,b) diluent denoted by number was replaced with fresh diluent for the first time (b) after 76 hr. of storage, (B) after 150 hr. of storage, (C,c) same as (B,b) except that 100 mg. % calcium carbonate was added to the fresh diluent.

rate of sperm in this particular diluent. Table 1 shows that 70% of the initial number of live sperm in this sample were dead at the end of 76 hr. of storage, as compared to only 13% dead in the coconut milk diluents 1a and 2a. Evidently, acidity and deteriorative processes associated with senescence are the principal factors responsible for the depressed activity of sperm in Diluents 1a, 1A, 2a, and 2A. The results also clearly indicate that the chemical environment provided by the coconut milk diluent is far superior to KRBF in supporting and extending

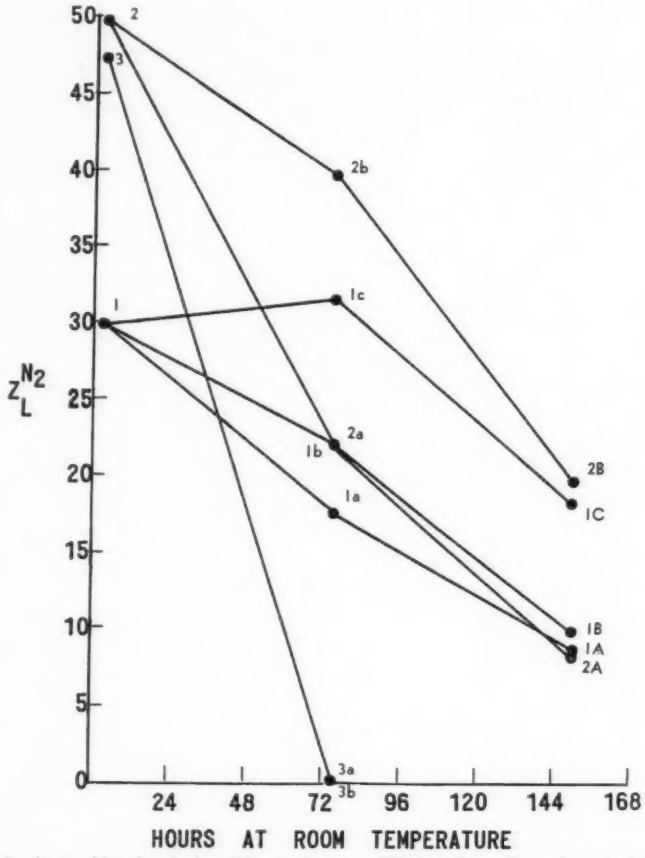

FIG. 2. Anaerobic glycolysis of bovine sperm. Explanation of numbers and letters associated with curves, mean motility, pH range, and per cent live sperm same as in Figure 1.

the livability of sperm. The influence of pH per se is further indicated by a comparison of 76-hr.-old spermatozoa in Diluents 1a and 2a. Since the age and mortality rate is essentially the same for these sperm, the greater degree of inactivation observed in Sample 1a is ostensibly correlated with its higher hydrogen ion concentration.

Reversibility of acid inhibition. Table 1, as well as Figures 1 and 2, reveals that the inhibited respiration, anaerobic glycolysis, and motility of sperm, stored in unchanged diluents for 76 hr. (1a, 2a) and 150 hr. (1A, 2A) at room temperatures, were partially and in one instance (1c, Figure 2) completely reversed by resuspending the aged, metabolically depressed sperm in fresh diluents having a higher pH. Sperm maintained in unchanged Diluent 1a for 76 hr. retained 55,

59, 50, and 85% of their initial values for oxygen uptake, lactate production, motility, and live sperm, respectively. When these acid-inhibited sperm were reactivated by resuspension in fresh CMC (1b), their oxygen consumption, carbon dioxide production, and motility were increased by 27, 25, and 74%, respectively. Replacing the diluent in 1a with fresh CMCC (1c), thereby making the basicity of the medium surrounding these sperm greater than in 1b, further enhanced the recovery of metabolic activity. The reactivated sperm in 1c increased their respiration, anaerobic glycolysis, and motility by 40, 80, and 100%, respectively. The rise in O_2 uptake, and in CO_2 production, approached statistical significance, whereas the increased motility was statistically significant ($P < .05$). An essentially similar recovery pattern was recorded for reactivated sperm initially treated with CMCC (2b). According to the results, the almost complete loss of sperm activity in 76-hr.-old KRBF diluent (3a) was due to the extremely low survival rate of sperm stored in this medium. Recovery after realkalinization of this sperm suspension (3b) was barely measurable.

The data collected after 150 hr. of storage suggest that the sperm in unchanged Diluents 1A and 2A have been maximally inhibited. This was manifested by the marked curtailed production of acid metabolites, low rates of respiration and glycolysis, complete cessation of motility, and the relatively low mortality rate of these sperm. Ostensibly, the low metabolic activity exhibited by the acid-inactivated sperm at this time represents the basal energy level required to maintain livability. Sperm activity in 1A after six days of storage at room temperature was 21, 30, 71, and 0% of the initial values for O_2 consumption, CO_2 production, live sperm, and motility, respectively. Reactivation by increasing the pH of the sperm medium (1B), raised the values for respiration, glycolysis, live sperm, and motility by 40, 33, 81, and 50%, respectively. Resuspending the inhibited sperm in the more alkaline CMCC (1C) elevated these parameters of metabolic activity by 47, 61, 82, and 75%. Resuspension of acid-inhibited sperm (2A) in fresh, alkaline CMCC media after 150 hr. of storage at room temperatures reversed inhibition to an extent similar to 1C.

An interesting paradoxical fact was the appearance of an increased number of live sperm in post-storage reactivated samples 1C and 2B (Table 1). Apparently, a number of inhibited, viable sperm, after storage in aged diluent take on the permeability characteristics of dead sperm. It must be assumed from these results that the staining technique is not a completely reliable method for differentiating live-dead sperm.

DISCUSSION

Although the factors which influence sperm longevity are extremely complex, the present evidence suggests that the in vitro life-span of bovine spermatozoa is within certain limits inversely proportional to sperm activity. Presumably, any physical or chemical agent which reversibly reduces metabolic activity can extend the livability of these sperm. The results of this investigation indicate that in a pH range of 5.58–5.80 bovine sperm motility, respiration, and glycolysis are

reversibly and maximally inhibited. This effect of acidity on sperm movement is in accord with the earlier observations of Koelliker *(9)*, who noticed that a slightly acid solution might inhibit sperm motility and that reactivation followed upon neutralization of the acidity; and with the work of Gunther *(7)*, who came to the conclusion that mammalian sperm activity is a function of the hydrogen ion concentration. The work of R. S. Lillie *(17)* on frog sperm and of Gray *(6)*, Cohn *(4)*, and F. R. Lillie *(16)* on echinid sperm verified the fact that these sperm respond in a similar manner to hydrogen ions. The present conclusion, that sperm respiration and glycolysis are directly correlated with pH, also is in agreement with the literature *(11, 14, 27)*, and is consistent with the currently accepted view that pH may cause an ionization at the active site of an enzyme which would modify its catalytic activity *(20)*.

The observed progressive increase of lactic acid, produced by stored spermatozoa suspended in a medium poor in oxygen and rich in glycolyzable substrate, was undoubtedly responsible for lowering the pH and the catalytic activity of one or more enzymes. Apparently, the rate-limiting effect imposed by acid-inhibited enzymes in the metabolic cycle resulted in a depression of respiration, glycolysis, and motility. As long as the pH did not fall below 5.5, the inhibitory action of excessive amounts of lactic acid was reversed by alkalinizing the sperm medium. The gradual decline in recoverability observed over the 150-hr. storage period is attributable to sperm senescence and to the toxic deteriorative changes in the suspension media. Apparently, below a pH of 5.5 irreversible enzyme denaturation occurs, and the excess hydrogen ion becomes lethal to the sperm. This is in close accord with Emmens' results *(5)*, which indicated that a pH below 5.8 was spermicidal. Emmens also noted that spermatozoa stored at room temperatures and rendered immotile by acidity could have their motility restored by alkalinization, provided they had not been too long (several hours) at an adverse pH. This is in striking contrast to the present results, which conclusively demonstrated that acid-inhibited sperm kept at room temperatures can be maintained in a viable state for several days. An appreciable amount of recovery was obtained when these inactivated sperm were alkalinized by resuspension in fresh CMC or CMCC media.

It seems probable that the ability of lactic acid–inhibited sperm to remain in a livable state at room temperatures for an extended period of time is partly a function of the physico-chemical environment (besides pH) provided by the sperm diluent. Rapid denaturation of some of the constituents in commonly used artificial and synthetic diluents is probably instrumental in lowering the survival rate of metabolically inhibited sperm kept in these diluents. In this respect, the coconut milk–sodium citrate medium employed in this study is a particularly suitable diluent, since it adequately supported the viability of inactivated sperm by preserving the functional integrity of the blocked enzymes and by forestalling rapid and destructive physico-chemical changes.

The choice of calcium carbonate as a reactivating agent was made after a

number of preliminary trials indicated it was less toxic than sodium, potassium, or magnesium carbonate. The toxic effect of calcium on sperm activity *(11)* was conveniently prevented by the complexing action of the citrate in the medium used in the present study. More recent work has shown that realkalinization by renewal of the medium can be avoided by adding proper amounts of solid base directly to the aged sperm suspension. Exploratory trials with the buffer Tris (hydroxymethyl) aminoethane (Sugma 121) suggests that it has considerable promise as a reactivator which can be added directly to the medium in solid form.

It would appear from the findings of this investigation that reversible acid inhibition can provide an economically usable number of sperm for the purpose of artificial insemination after several days of storage at room temperatures. This conclusion is predicated on several laboratory criteria which purportedly reflect the functional capacity of spermatozoa. It is anticipated that results on the fertilizing capacity of acid-inactivated sperm, as indicated by extensive field trials, will be presented in the near future.

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TECHNICAL NOTE

EFFECT OF INCUBATION VESSEL ON THE RECOVERY OF $C^{14}O_2$ DURING INCUBATION OF BULL SPERMATOZOA WITH GLYCEROL-1-C^{14} [1,2]

A report by Salisbury and Sharma *(6)* showed that oxygen uptake of bull spermatozoa diluted in 0.9% saline varied directly, but not proportionately, with the ratio of the exposed surface area-to-volume (S/V) of the fluid in the incubation vessel. Maximum oxygen uptake was obtained with an S/V ratio in the range of 1–1.9. A series of experiments investigating the uptake and metabolic utilization of glycerol -1-C^{14} by frozen and unfrozen bull spermatozoa has been conducted in this laboratory *(4, 5)*. In the course of these experiments it was found that radioactive $C^{14}O_2$ recovery during incubation of spermatozoa varied with the type of incubation vessel employed.

Therefore, an experiment was designed to investigate the following incubation techniques: *(a)* 125-ml. Erlenmeyer flasks vs. 20- by 180-mm. test tubes as incubation vessels, and *(b)* incubation at 5° C. for 18 hr. vs. 37° C. for 3 hr. The vessels were not agitated during incubation. Spermatozoa (3.5×10^9 from pooled ejaculates) were washed twice with 0.9% saline and resuspended in 5 ml. of 0.9% saline containing 1% glycerol by volume and 5μc. of glycerol-1-C^{14}. Aliquots for chemical analysis were removed initially and at the end of the incubation period and were deproteinized with 10% trichloroacetic acid. Filtrates were analyzed for sugar by a modification of the anthrone method *(2)* and for lactate by the method of Barker and Summerson *(1)*. All treatments were prepared in duplicate. The samples were incubated under nitrogen and contained 1,000 units of penicillin G and 1,000 μg. of streptomycin sulfate per milliliter of diluent to prevent bacterial interference. The procedures for recovery and assay of $C^{14}O_2$ and for measurement of sperm uptake of glycerol-1-C^{14} have been described previously *(3, 5)*. Control samples containing spermatozoa inactivated with one drop of 10 *N* sulfuric acid were incubated in flasks for 3 hr. at 37° C. in each trial.

As shown (Table 1), irrespective of the incubation vessel used, incubation procedures of 3 hr. at 37° C. and 18 hr. at 5° C. affected both the radioactivity of the CO_2 and the uptake of glycerol-1-C^{14} by the spermatozoa. Sperm incubated for 3 hr. at 37° C. produced 62% more $C^{14}O_2$, but showed 23% less uptake of glycerol-1-C^{14} than did those incubated for 18 hr. at 5° C. Thus, there was less metabolic utilization of glycerol-1-C^{14} by sperm incubated at 5° C. than at 37° C., even though the incubation period was six times longer.

In both Tables 1 and 2, the inactivated spermatozoa in the control samples did not produce a significant amount of $C^{14}O_2$. However,

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TABLE 1

Comparison of two types of incubation vessels and two incubation procedures on the uptake and metabolic utilization of glycerol-1-C^{14} by bull spermatozoa

(Average of two replicates)

Measurement[a]	Incubation vessel	Control	Incubation for: 3 hr. at 37° C.	Incubation for: 18 hr. at 5° C.	Ratio: 3 hr. 37° C. / 18 hr. 5° C.
$C^{14}O_2$	Flask	4	352	132	2.67
	Tube		159	60	2.65
Ratio	Flask / Tube		2.21	2.20	
Sperm uptake	Flask	139	377	496	0.76
	Tube		386	500	0.77
Ratio	Flask / Tube		0.98	0.99	

[a] Counts per minute per milligram of $BaC^{14}O_3$; counts per minute per 10^9 sperm.

TABLE 2

Effect of incubation on the uptake and metabolic utilization of glycerol-1-C^{14} and glycolytic activity of bull spermatozoa during incubation for 3 hr. at 37° C.

(Average of four replicates)

Incubation vessel		S/V[a] ratio	Specific activity of $C^{14}O_2$	Glycerol uptake	Sugar loss	Lactate gain
			(c.p.m./mg $BaCO_3$)	*(c.p.m./10^9 sperm)*	*(μg/10^9 sperm)*	*(μg/10^9 sperm)*
Flask		6.64	280[b]	371	42	33
Tube		0.63	127	356	26	66[c]
Ratio	Flask / Tube	10.54	2.20	1.04	1.62	0.50
Control flask			3	125	14	2

[a] Surface area–to-volume ratio.
[b] $P < 0.01$.
[c] $P < 0.05$.

there was some glycerol-1-C^{14} associated with the dead sperm after four washings with physiological saline. The specific activity of CO_2 from semen samples incubated in test tubes was only 45% of that obtained from samples incubated in Erlenmeyer flasks. This was true, irrespective of the incubation procedure used. In Table 2, the difference in the specific activity of $C^{14}O_2$ of 153 counts per minute in favor of flasks over tubes was highly significant ($P < 0.01$) as determined by the "*t*" test. However, there was no significant difference in uptake of glycerol-1-C^{14} by sperm in the two types of vessels. A calculation of the approximate amount of CO_2 recovered from the two types of vessels showed that only 60% as much was recovered from test tubes as from flasks (60 and 99 μg., respectively).

The S/V ratio, calculated by the method of Salisbury and Sharma *(6)*, was 10.5 times greater in the flasks than in the test tubes and undoubtedly resulted in less gaseous exchange at the exposed surface of the fluid in the test tubes, thereby reducing the amount of $C^{14}O_2$ recovered during the incubation period. The data in Table 2 for sugar loss and lactate gain show that twice as much lactate ($P < 0.05$) accumulated in the test tubes as in the flasks, whereas only 38% as much sugar was utilized. Thus, it appears that the larger S/V ratio contributed to the maintenance of a more aerobic condition in the flasks than in the test tubes, thereby resulting in a more rapid turnover of lactate formed from glycerol-1-C^{14} to $C^{14}O_2$.

These results confirm those of Salisbury and Sharma *(6)* and clearly show that in metabolic studies depending upon measurements of gaseous exchange, careful consideration should be be given to the surface area–to-volume ratio existing in the vessel containing the cell suspension.

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OUR INDUSTRY TODAY

SOME METHODS OF EVALUATING THE PRODUCTIVITY OF LABOR IN MILK PLANT OPERATIONS [1]

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Labor cost is a major item of expense in fluid milk processing plants. This cost varies greatly among different plants, because of factors such as numbers of products and packages, differences in plant layout, and equipment and employee efficiency. These differences in plants make it very difficult to use a common yardstick for measuring their relative efficiency.

Since previous studies *(1)* showed that the work-sampling technique gave results statistically comparable to production studies with much less time involved, it was decided to use this technique to obtain information that would be helpful in comparing the relative efficiency of some operations in fluid milk plants.

Some of the major considerations of this study are: *(1)* to measure man-minute requirements for the various operations associated with fluid milk handling in a dairy plant, using the work-sampling technique; *(2)* to determine any differences in productive and nonproductive time that may be inherent in a specific job; *(3)* to determine the functional efficiency of a dairy plant by comparing established standard times to the actual time required to perform the various functions; *(4)* to evaluate the differences in efficiency that exist between plants for the receiving, processing-packaging, and packaging operations.

METHODS

Several North Carolina milk plants ranging in size from 7,000 to 25,000 lb. daily volume were selected, in which the work-sampling technique could be used to determine the utilization of labor. The procedure followed was to contact the plant manager and become acquainted with the plant layout, plant employees, and plant equipment. The study was begun on Monday morning and carried through the following Saturday, to complete a weekly cycle. The observation round was begun on the first employee to be observed when the watch indicated 5 min. from the previous reading. A check-sheet previously had been prepared which included the activities for each employee for the observations to be recorded. As the observation was made, the activity for the specific function was checked on the check-sheet. Thus, at the end of each day, a complete record of the employee's activities was shown on each employee's check-sheet. Employee efficiency was calculated by totaling the observations on an employee, and determining how many were productive. The per cent efficiency was calculated by dividing the number of productive readings by the total observations.

Standard times were applied to each day of plant operation. Standard times were taken from previous time-study work at North Carolina State College *(1–6)*, or times developed by the authors. These data were applied each day to the plant's receiving, process-packaging, and packaging operations. Thus, a potential or theoretical time was determined for each operating day. The per cent efficiencies were determined by dividing the man-minutes theoretically required to perform a function, as established by standard times, by the man-minutes actually required to do the function.

Analysis of the functional data (i.e., bottle filler function, separator function, etc.) was necessary in order to make comparison between plants. These data were converted into man-minutes per 100 lb. of dairy product and man-minutes per package. Both conversions were necessary to show the complete picture of plant efficiency.

The man-minute requirements were broken down into the receiving, processing-packaging, and packaging operations. Since the employees shifted between the jobs of processing, packaging, and stacking in many of the plants, it was thought that a better comparison could be made of labor requirements if a broad category such as processing-packaging was used. Also, packaging required such a high percentage of the labor that an attempt was made to isolate and study this particular operation separately. The receiving operation included those activities required in the handling of the raw milk from the time it was placed on the conveyor in cans until it was transferred to storage tanks or pasteurizing vats. The processing-packaging operation began with the raw milk delivered to storage tanks or to the pasteuriz-

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ing vats, and ended with stacking of filled cases in the cold storage room. It encompassed processing and packaging operations plus delay time. The packaging operation began with the delivery of the processed product to the filler and ended with the placing of the filled packages in the empty crates. It did not include delay time.

"With-delay" values for all operations were computed from times that included all delay time inherent to the operation of a particular plant, i.e., waiting for producer milk. "Without-delay" values were calculated from the net productive times required to perform the operation.

The error term in the analysis of variance and covariance was obtained by the difference between days within a plant.

RESULTS

A description of the labor force, products manufactured, and output of the seven plants is presented (Table 1).

Job productive time. Table 2 shows the mean productive time of six jobs in the plants. An analysis of variance *(9)* was carried out on the data. The difference in productive time between plants was significant at the 1% level for glass filler, paper filler, utility, and receiving employees, while the difference in productive time for the bottle washer and storage room employees was significant at the 5% level.

An analysis of variance was carried out, to determine the significance of differences in productive time on jobs within a plant. The differences in productive time in jobs within a plant were significant at the 1% level for Plants B, C, E, and F, and significant at the 5% level for Plants A and F. The differences in productive time in Plant D were not significant, probably due to the large variation of productive time from day to day.

Some of the differences between the productive times of the six jobs can be attributed to: *(1)* the content of the job; and *(2)* the initiative and ambition of the individual employee.

Function efficiency. Table 3 shows the efficiency of six functions in the plants. It was necessary for a function to contain approximately 100 man-minutes or more before the function was considered for statistical analysis, since smaller values usually would show excessive variations.

An analysis of variance was carried out on the data in Table 3. to determine the significance of differences in efficiency in the functions between plants. The differences in the efficiency of the functions between plants were significant at the 1% level on all the functions compared. The variations in the amount of time devoted to the different functions are believed to be accounted for mainly by one or more of the following: *(1)* type, amount, and capacity of equipment used; *(2)* mechanical condition of equipment; *(3)* scheduling of operations; *(4)* work methods; *(5)* plant layout; *(6)* the volume of product handled and package size used, and *(7)* management's attitude on plant operations and procedures.

Man-minute requirements. The man-minutes required per 100 lb. of product and per package before and after adjusting for differences in package sizes in the plant operations are shown (Table 4). Since one obvious reason for plant-to-plant variation in these numbers is the difference in distribution of packaged prod-

TABLE 1

Comparative data on labor force, number of products, and different sized packages, operating speed of fillers, and average number of units packaged daily

Plant	No. of employees for processing-packaging operation	No. of products	No. of different sized packages for all products	Average number of units packaged daily						Operating speed of fillers			
				Glass			Paper			Packages per minute			
				Glass			Paper			Glass		Paper	
				½-pt.	pt.	qt.	½-pt.	pt.	qt.	½-pt.	qt.	½-pt.	qt.
A[a, b]	6	4	5	12,776	0	303				71	48		
B[a]	10	9	17	2,827	723	3,805	1,357	326	3,758	38	38	20	20
C[c]	8	8	17	2,162	1,251	5,750	922	564	1,586	48	42	28	28
D	8	9	19	3,940	989	3,804	791	529	1,424	55	48	20	20
E[a, c]	8	11	21	3,895	1,259	3,259	734	620	4,466	49	35	20	20
F[b]	6	8	14				6,593	2,880	7,024			57	57
G	6	8	14				505	399	4,705			20	20

[a] Plants A, B, and E operated on a four-day week schedule.

[b] Plants A and F used H.T.S.T. pasteurization, whereas the remainder of the plants used vat pasteurization.

[c] Plants C and E used a straight-away can washer, whereas the remainder of the plants used rotary can washers in the receiving operation.

TABLE 2

Comparison of mean productive times of jobs in the plants

Plant	Per cent productive time						
	Glass filler employee(s)	Paper filler employee(s)	Bottle washer employee(s)	Storage room employee(s)[a]	Utility employee(s)[b]	Receiving employee(s)	Plant employees[c]
A	87.5		81.4	82.2	85.9	87.9[d]	85.0
B	96.3	97.5	87.2	78.7	85.6	85.0	90.0
C	89.3		81.2	80.8	88.7	76.8	85.3
D	88.6	83.3	73.5	79.4	80.9	79.9	81.0
E	91.4	98.4	83.8	82.6	91.1	91.1	90.1
F		85.9		69.7	89.9	77.0	83.6
G		95.5			95.6	91.0	94.3

[a] Consisted of stacking filled cases and assisting on fillers.
[b] Utility employees perform such jobs as assisting with various functions, and disassembling, cleaning, and assembling of equipment and sanitary lines.
[c] These values represent a composite weighted mean of all employees who contributed to the receiving, process-packaging, and packaging operations over the period studied.
[d] Plant A received milk in tank trucks.

uct among quarts, pints, and half-pints, the values are also given adjusted to a common number of quarts, pints, and half-pints. The adjustment was made by the multiple covariance technique *(9)*.

An analysis of variance was carried out on the receiving data (in Table 4), to determine the significance of differences in the man-minutes required per 100 lb. of product received in the seven operations. The differences in the man-minutes, "with-delay" and "without-delay," required per 100 lb. of product, were significant at the 1% level.

The variations of man-minutes required to receive milk in the seven plants were caused by several factors; namely, *(1)* the volume of milk received; *(2)* the type and capacity of the equipment; *(3)* the number of employees in the receiving room, and *(4)* the amount of delay time lost waiting for milk to arrive from producers.

An analysis of covariance was carried out on the data in Table 4, to determine the significance of differences in the man-minutes required per 100 lb. and per package of product before and after adjustment for different package sizes in the seven processing-packaging operations. The differences in the man-minutes required per 100 lb. and per package of product produced were significant at the 1% level in all comparisons.

Some of the factors responsible for the differences in labor requirements in processing-packaging between plants were: *(1)* differences in plant layout; *(2)* difference in type and capacity of equipment; *(3)* supervision of employees; *(4)* scheduling of work; *(5)* work methods; *(6)* volume of product handled and package sizes used; *(7)* management's attitude on plant procedures and operations; *(8)* initiative of employees, and *(9)* incentive offered by management.

The labor requirements for the packaging operations were figured on the man-minutes required per package of product. The calculations were made only on the "without-delay" basis, because it was difficult to determine whether a delay by an employee in the processing room should be charged to the processing-packaging

TABLE 3

Comparison of mean per cent efficiency of six functions in the plants[a]

Plant	Per cent efficiency					
	Glass filling function	Paper filling function	Bottle washer function	Sanitary pipe function	Vat function	Receiving function
A	52.3		84.4	55.0		
B	39.1	59.7	57.4	36.6	33.9	55.7
C	62.0		78.0	63.7	61.6	95.9
D	59.4	65.4	51.3	75.7	60.5	92.7
E	80.0	84.4	61.6	65.7	63.9	79.5
F		84.5		67.4		104.6
G		57.7		41.8	28.62	57.8

[a] Per cent efficiency $= \frac{\text{theoretical time (standard time)}}{\text{actual time}} \times 100.$

TABLE 4

Comparative mean values for man-minutes required per 100 lb. and per package before and after adjusting the plants for differences in package sizes in the receiving, processing-packaging, and packaging operations

Plant	Man-minutes required per 100 lb. of product						Man-minutes required per package of product					
	Receiving operation		Processing-packaging operation				Process packaging operation			Packaging operation		
										Glass	Paper	
	Without delay	With delay	Without delay	With delay	Without delay (adjusted)	With delay (adjusted)	Without delay	With delay	Without delay (adjusted)	With delay (adjusted)	Without delay	Without delay (adjusted)	Without delay	Without delay (adjusted)
A	1.4	1.6	18.6	23.4	19.4	26.2	0.11	0.14	0.12	0.18	0.03	0.04		
B	2.0	2.4	14.1	16.0	15.4	16.8	0.22	0.25	0.24	0.27	0.11	0.10	0.18	0.14
C	1.3	1.7	10.7	12.5	10.3	11.3	0.17	0.20	0.17	0.18	0.05	0.05	0.13	0.15
D	1.1	1.5	13.1	16.6	12.4	15.7	0.19	0.24	0.18	0.22	0.05	0.05	0.11	0.12
E	1.7	1.8	10.2	11.7	11.0	13.0	0.15	0.17	0.16	0.17	0.05	0.06	0.09	0.01[a]
F	1.1	1.2	7.2	8.8	7.3	9.1	0.09	0.12	0.09	0.10			0.05	0.22[a]
G	2.0	2.2	13.4	14.3	11.8	11.3	0.25	0.27	0.25	0.24			0.12	0.02[a]

[a] These values are not too reliable, due to difficulties in the adjustment procedures. (Explained in the discussion of the packaging operation.)

or packaging operation. Therefore, the decision was made to analyze the function "without-delay" time included, to remove this possible error.

The adjusted paper packaging values for Plants E, F, and G were far out of line as compared with the adjusted values for the other plants in the glass packaging and the processing-packaging operations. A reason for this may be the very unusual distribution of type of products packaged in these plants. Plants E and G had many more quarts and far less half-pints than the other plants; whereas, Plant F had a very high volume of both quarts and half-pints. The covariance adjustment procedure essentially adjusts each value by an amount proportional to the difference between the number of quarts, pints, and half-pints for the specific plant, and the average number of quarts, pints, and half-pints for all the plants combined. For Plants E, F, and G these differences were apparently beyond the range where a simple proportional adjustment is adequate. Thus, one would hesitate to put much faith in their adjusted values.

The four main factors responsible for the differences in man-minutes required per package in the glass and paper packaging operations are *(1)* differences in type and capacity of bottle-filling equipment; *(2)* differences in plant layout; *(3)* differences in volume of product handled and package sizes used, and *(4)* differences in methods.

DISCUSSION

The procedure used in this study is believed to be a valid method of evaluating the efficiency of a fluid milk operation. It has some advantages over some other procedures, since the method of collecting data seems well adapted for use in the industrial dairy plant. Also, several efficiencies figures can be determined from the data, thus giving more facts and information about the plant operation upon which to base decisions.

Several types of efficiency information are obtained on analyzing the data from a work-sampling study. Employee efficiency shows what per cent of the time an employee is contributing to a productive function, although it does not indicate the speed of work. Functional efficiency evaluates a function by established standard times, or it shows the time that should go into performing a job compared with the actual time consumed in completing the job. Man-minute requirements per 100 lb. and per package for the receiving, processing-packaging, and packaging operations are useful in studying the relative position a plant occupies in the efficiency of these operations.

Man-minute requirements per 100 lb. and per package are certainly important in evaluating

the efficiency of a plant, since these figures can be transformed into cost figures by applying the dollars per hour paid for labor. On the contrary, a plant should not be diseriminated against in an efficiency study just because it does not have the facilities of another plant. For example, it is inevitable that Plant F will have a lower man-minute requirement, since the type and size of equipment are far superior to those in the other plants. Again, in Plant A, with 98% of its volume in half-pints, a low man-minute requirement per package is expected. Plant G, with 80% of its volume in quarts, is expected to have a relatively low man-minute requirement per 100 lb. Adjustments for differences in the number of half-pints, pints, and quarts between plants were performed to reduce the influence of this important variable. Perhaps more study of this technique would result in better adjustment factors and a more accurate basis for comparing plants.

Employee efficiency, functional efficiency, and man-minute requirements are valuable tools in determining labor utilization; however, certain limitations are present which should be noted in the application of these determinations. Employee efficiency, which is useful in showing the time a worker is contributing productively to a function, does not take into consideration some other important factors. Some of these are speed of work by the employees, methods, and type and capacity of equipment. However, the validity of this determination is related to the fact that, if all conditions were constant between plants, the man-minutes required would vary directly with the productivity of the workers. Functional efficiency takes into account the factors mentioned above, since this determination relates the actual time consumed in completing a function to an established standard time. A plant must be using good methods and equipment for a high functional efficiency figure to be obtained. The efficiency of the employees is also related to the functional efficiency; however, because a plant's employees have a high productive time does not necessarily mean that the plant is functionally efficient, since the employees in one plant may be working more productively than those in another plant. If a plant can combine a high employee efficiency with a high functional efficiency, a relatively low man-minute requirement can be obtained.

No single efficiency determination should be taken as an end in itself; however, with several determinations available, an individual who is familiar with the plants should be able to draw valuable conclusions about the efficiency of operation of a particular plant.

Management should have the information given above, in order to make sound decisions. However, the most economical balance must be found between the facts obtained and the cost involved. The techniques used for obtaining labor utilization data in this study are believed to be sound and relatively inexpensive.

SUMMARY

A significant difference was found between plants in employee efficiency as related to the job performed by a worker. The per cent efficiency was calculated by dividing the number of productive observations by the total observations.

A significant difference was found between plants in functional efficiency, which is determined by dividing the theoretical time as established by standard times by the actual time consumed in the completion of the function.

Analysis of labor utilization by man-minutes required per 100 lb. and per package for the receiving, processing-packaging, and packaging operations in the plants included in this study indicated that a significant difference existed between plants.

No single efficiency determination alone can be taken as a measure of the relative efficiency of fluid milk plants. It is believed that the best evaluations can be reached by using the information provided by employee efficiency, function efficiency, and man-minutes per package and per 100 lb.

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WORK-SAMPLING AS A TECHNIQUE IN DETERMINING LABOR UTILIZATION OF DAIRY PLANTS [1]

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The dairy industry must seek means whereby it can lower costs and, at the same time, maintain high quality of product. To meet the rising costs incurred in an inflated economy, it is necessary either that the selling price of the product be increased or that a more efficient operation be accomplished, whereby the margin of profit may remain favorable. Following the principles of economics, the former method would lower the sales of dairy products and put the industry in a less favorable position than it is today. The latter method seems to be the preferable solution to the problem, and this appears to be the conclusion that large corporations have reached in their struggle for survival in this era of mass production.

Several reports on the processing cost in dairy plants have revealed that labor is the greatest single item of expense. Connor, Spencer, and Pierce *(2)*, in a precise economic study of a hypothetical dairy plant, pointed out that 59.9% of the total costs could be attributed to labor costs. Therefore, the logical place to begin in lowering costs would be to increase the efficiency of plant labor.

To increase the efficiency of labor in an operation, it is of major importance to know its present status and to compare this with a known standard. The status of labor may be obtained through work-sampling, which is a random-observation, time-study technique. The comparison may be made through standard times, which are pre-established times whereby the time necessary to accomplish a unit of work is determined.

By making comparisons of the actual time required for the various functions, as found through work-sampling, with the theoretical time obtained by applying standard times to the operation, the dairy plant manager is in a position to draw valid conclusions about the operation. If the decision is reached that a change in the operation or some other corrective action is necessary then, at some later date, the studies can again be made to check the effect of the corrective action.

Work-sampling, ofter referred to as Ratio-Delay or Observation-Ratio, is a method for which an increased number of uses is being found. Work-sampling constitutes a tool whereby actual working items of dairy plant functions can be obtained.

Tippett *(6)*, in the English textile industry, searching for a better way to determine delay and variation allowances, described a new method of ratio-delay study. In making a ratio-delay study, a large number of observations was taken at random intervals, to record whether a machine was operating. Tippett stated that the percentage number of readings that record the machine as working will tend to equalize the percentage time it is in that state. Likewise, if random observations are taken of the operator's cycle at work, the percentage of readings recording the operator as performing a certain operation or group of operations is an estimate of the percentage time spent in those operations. If the readings are randomly distributed over a sufficiently long time, this relationship holds, whether the machine stoppages or operations of the operators are short or long, many or few, regular or irregular. Tippett also stated that unless exceptional precautions are taken, the total error is not likely to be reduced much below about 2%, except when the percentage measured is very high or very low (i.e., between 0 and 5% or between 95 and 100%), or when comparisons are being made under conditions in which systematic errors are constant.

Under the direction of Morrow *(4)*, the ratio-delay method of Tippett was put to practical test in three separate industries. These studies showed the new method to be a statistical technique, highly practical and productive of more accurate results at less cost than methods previously employed.

Allderige *(1)* developed statistical alignment charts for practical use in work-sampling. The use of alignment charts makes it possible to eliminate the use of formulas.

Another advantage of using this random observation technique is that man-power efficiency of plant employees can be determined in addition to labor utilization. This type of knowledge leads to better use of available man-power, provides a basis for judging efficiency, and helps in estimating labor requirements and work-load distribution. In addition, information on labor utilization provides a basis for the formulation of a wage-incentive payment plan.

Since no data were available on the use of work-sampling in the dairy industry, it was necessary to make a study to illustrate its usefulness, also to demonstrate the ability of the time-study man to utilize this tool as a time-study aid.

[1] Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station, Raleigh, as Paper No. 870 of the Journal Series.

METHODS

A production and a work-sampling study were run concurrently on labor and machine utilization in the North Carolina State College Dairy Plant. The production study consisted of one in which the time-study man made one or more all-day time studies on a worker, recording what the worker did and the time necessary for the completion of the distinct jobs accomplished. The work-sampling data were collected, with observations being made every 5 min. The study was carried out on a general helper, the glass bottle filler operator, and the glass bottle filler. The general helper was observed over a five-day period and the glass bottle filler operator over a four-day period. The observations on the glass bottle filler machine were made over an eight-day period. A stop watch was used to determine when the work-sampling observation was to be made. A check sheet that covered any job that might be performed was provided to classify the activity.

RESULTS AND DISCUSSION

The results of the comparison between the production and work-sampling study on a general helper are presented (Table 1).

From the results shown, it should be noted that the maximum difference between the two techniques by days varied from −3.9% on Day One to 3.8% on Day Three, when the productive time on the production study technique is compared with the productive time of the work-sampling technique. After applying statistical analysis to the data, it was found that the difference between the production and work-sampling techniques was not significant, since it exhibited a "t" value of 0.70; a value of 2.78 would be necessary to show a significant difference between the techniques. The 95% confidence interval for the difference was found to be −0.88% ± 3.59%, or it might be said that the true difference lies in the range from 2.71 to −4.47%.

TABLE 1

Comparison between a production and work-sampling study on a general helper

Day	Production study: Productive time	Work-sampling study: Productive time	Difference
	(%)		
One	80.5	84.4	−3.9
Two	88.2	90.0	−1.8
Three	86.9	83.1	+3.8
Four	76.1	78.4	−2.3
Five	79.6	80.4	
Weighted mean	82.72	83.59	−0.87

[a] $t_{05} = 0.70$ for significance = 2.78.

Confidence interval for unweighted average difference = −0.88% ± 3.59%, or 2.71 to −4.47%.

The results of the comparison between the production and work-sampling study of a glass bottle filler operator are represented in Table 2.

Statistical analysis on the data of Table 2 shows that the difference between the production and work-sampling techniques was not significant. A "t" value of 3.18 is necessary for significance and the "t" value for these data was 0.10. The 95% confidence interval for the difference was found to be −0.025% ± 0.795%, or the true difference between the techniques lies in the range from 0.77 to −0.829%.

TABLE 2

Comparison between a production and work-sampling study on a glass bottle filler operator

Day	Production study: Productive time	Work-sampling study: Productive time	Difference
	(%)		
One	89.9	90.0	−0.1
Two	86.0	86.5	−0.5
Three	93.6	93.5	+0.1
Four	90.5	89.8	+0.7
Weighted mean	90.14	90.18	−0.04[a]

[a] $t_{05} = 0.10$ for significance = 3.18.

Confidence interval for unweighted average difference = −0.025% ± 0.795%, or 0.77 to −0.82%.

The results of the comparison between the production and work-sampling study on the glass bottle filler machine are presented (Table 3).

The "t" value of 0.67 for these data indicated that there is no significant differences between the techniques. The 95% confidence interval for the difference was found to be −0.55% ± 1.94%, or the true difference between the techniques lies in the range from 1.39 to −2.49%.

Thus, the results for these studies indicated that the accuracy of collecting data by the work-sampling technique was comparable to the production-study technique. Comparison studies between these techniques were made in several industries by Morrow *(4)*, Petro *(5)*, and MacNiece *(3)* and, in each case, the techniques gave comparable results.

SUMMARY

Work-sampling was found to be a useful time-study technique in securing labor utilization data in a dairy plant.

The work-sampling technique has several advantages over other time-study procedures in the collecting of data on labor requirements.

TABLE 3

Comparison between a production and work-sampling study on a glass bottle filler machine

Day	Production study	Work-sampling study	
	Productive time	Productive time	Difference
	(%)		
One	75.6	79.7	−4.1
Two	63.9	61.6	+2.3
Three	66.0	66.3	−0.3
Four	67.4	66.7	+0.7
Five	69.6	69.6	0
Six	65.6	70.0	−4.4
Seven	71.3	69.7	+1.6
Eight	58.2	59.2	−1.0
Weighted mean	68.19	68.86	−0.67[a]

[a] $t_{86} = 0.67$ for significance = 2.36.

Confidence interval for unweighted average difference = −0.55% ± 1.94%, or 1.39 to −2.49%.

The technique will obtain a maximum of information in a minimum of time, which is important both from the standpoint of the more efficient utilization of the time-study man's time and the cost to the plant. One man can study the entire plant operation, or any particular phase of the operation that management desires. Another feature of importance is that this technique will not interfere, to any extent, with the daily routine of plant operation. Workers pay little attention to the time-study observer, since the observations are made by mere glances at the workers, rather than by direct concentration on a specific employee, as utilized in a production study.

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OUR ASSOCIATION

SURVEY OF DAIRY PRODUCTS–JUDGING CONTESTANTS. I. WHAT VOCATIONS DO DAIRY PRODUCTS–JUDGING CONTESTANTS FOLLOW AFTER GRADUATION?

COMMITTEE ON JUDGING DAIRY PRODUCTS, A.D.S.A.
C. J. Babcock,[1] L. R. Dowd, P. A. Downs, E. L. Thomas, F. G. Warren, J. J. Willingham, and G. M. Trout, *Chairman*

What happens to the dairy industry–trained graduate? Has he forsaken the area of his training? Are changes of occupation any greater among dairy industry trainees than among other agricultural college graduates? Is specific dairy industry training necessary in an era of automation? Data giving answers to these questions are scarce. Actually, to what extent the dairy industry loses its trained men by transfer of interest is not known.

The current interest in dairy industry training, and the availability of names of all contestants participating in the Collegiate Students' International Contest in the Judging of Dairy Products since 1930, prompted the Committee on Judging Dairy Products, A.D.S.A., to make a study of the vocational status of these men.

Historical

Studies in vocational agriculture in high school by Rougeau in 1958 *(2)* show that drop-outs and changes to other interests were the rule rather than the exception. In one survey in 1958 [Anonymous, *(1)*], the percentage of drop-outs was appalling. Of 235 high-school graduates trained in vocational agriculture during a 20-yr. period, only 28, or 11.9%, were currently in the field of agriculture. Only 14 of the 235 graduates finished college. Of those 14, eight, or 57%, of them graduated in arts and sciences and in medicine—fields entirely unrelated to agriculture.

Stone in 1954 *(4)*, surveying 271 dairy graduates (production and manufacturing), found that 57.6% remained in the field of their major. Only three of 14 majors surpassed dairy in retention of their graduates in the field for which they were trained. These were: Food Technology, 20 students replying, 90%; Forestry, 715 students, 70%, and Landscape Architecture, 133 students, 64.7%. Sprague in 1956 *(3)*, examining careers of Agronomy alumni, listed 73 of 81 graduates, 90%, in 11 different types of employment, most of which were related, although some remotely, to farm crops and soils. Stone's *(4)* survey of 174 replies from Farm Crops majors showed that only 35.7% remained in that field, and that of 161 Soil Science major replies, 55.2% of them stayed with soils.

Scope of Study

The study involves 1,401 dairy products–judging contestants from 1930 to 1956, inclusive. Presumably, these contestants, by virtue of *a)* their specific training, *b)* contact with fellow trainees from other institutions, and *c)* enlarged vision of the dairy industry, through exposure to the dairy industry exhibitions and trade association meetings, were indoctrinated into dairy manufacturing. Therefore, in this study, it is assumed that these contestants are representative of the dairy industry graduates. A former study (Trout *et al.*, 1939) *(5)* showed that representatives from the body of contestants reflected a high degree of indoctrination into the dairy industry. The contestants in the current study represented 44 colleges and universities in 41 states and Ontario.

Study Procedure

The study was conducted with the full cooperation of all the dairy products coaches and/or dairy departments from which the contestants graduated. Lists of contestants, by years of participation in the Collegiate Students' International Contest in the Judging of Dairy Products, were mailed to the specific coach or department for checking and completion of information. Their mailing address and employment, especially, were sought. From these returned lists, percentage distributions, by geographical location of schools, were made.

Results

a) Distribution of present vocational status. Percentage distribution data, accounting for all contestants, particularly as to employment, are presented in Table 1. The data are most enlightening. They show that 73.8% of the contestants are employed in occupations either in or closely allied to the dairy industry. By geographic location of their Alma Mater, the figures range from 70.8% in the eastern to 78.3% in the western sections of the United States. Dairy plants account for approxi-

[1] Deceased.

TABLE 1

Accountability of dairy products–judging team–member graduates, 1930 to 1956, inclusive, with emphasis upon type of employment

Classification	Distribution of graduates from various sections of the United States									
	Central [a]		Eastern		Southern		Western		Total	
	(*No.*)	(%)	(*No.*)	(%)	(*No.*)	(%)	(*No.*)	(%)	(*No.*)	(%)
Dairy plant	With, or closely allied to, dairy industry									
Owner or co-owner	27	4.3	25	6.9	17	4.8	1	1.7	70	5.0
Manager or assistant manager	69	10.9	29	8.1	27	7.7	7	11.7	132	9.4
Superintendent or foreman	98	15.6	51	14.2	57	16.2	16	26.7	222	15.8
Miscellaneous (buyer, auditor, director, etc.)	8	1.3	0	0.0	0	0.0	1	1.7	9	0.6
Total	202	32.1	105	29.2	101	28.8	25	41.7	433	30.9
Education										
University professor	45	7.1	22	6.1	13	3.7	3	5.0	83	5.9
Vocational agriculture	13	2.1	3	0.8	2	0.6	1	1.7	19	1.4
County agricultural agents	5	0.8	5	1.4	6	1.7	0	0.0	16	1.1
Graduate studies	20	3.2	18	5.0	18	5.1	4	6.7	60	4.3
Other (undergraduate, experiment station, high school, veterinary medicine)	3	0.5	17	4.7	3	0.8	0	0.0	23	1.6
Total	86	13.7	65	18.0	42	12.0	8	13.3	201	14.3
Salesman										
Dairy products	19	3.0	10	2.8	8	2.3	1	1.7	38	2.7
Dairy machinery and supplies	40	6.4	9	2.5	10	2.8	2	3.3	61	4.4
Other (feed, seeds, farm fertilizer, meats, machinery, auto, tobacco, steel, etc.)	9	1.4	4	1.1	14	4.0	0	0.0	27	1.9
Total	68	10.8	23	6.4	32	9.1	3	5.0	126	9.0
Farming										
Dairy	13	2.1	16	4.4	16	4.6	3	5.0	48	3.4
General	25	4.0	5	1.4	12	3.4	3	5.0	45	3.2
Other (turkey, swine, beef, poultry)	6	0.9	0	0.0	0	0.0	0	0.0	6	0.4
Total	44	7.0	21	5.8	28	8.0	6	10.0	99	7.0
Fieldman	13	2.1	5	1.4	19	5.4	1	1.7	38	2.7
Total	13	2.1	5	1.4	19	5.4	1	1.7	38	2.7
Dairy laboratory										
Director	6	0.9	3	0.8	2	0.6	0	0.0	11	0.8
Technician and bacteriologist	11	1.7	5	1.4	0	0.0	0	0.0	16	1.1
Owner	1	0.2	1	0.3	0	0.0	0	0.0	2	0.1
Total	18	2.8	9	2.5	2	0.6	0	0.0	29	2.1
Departments of Agriculture and Health										
City	15	2.4	3	0.8	5	1.4	2	3.3	25	1.8
State	9	1.4	5	1.4	9	2.6	2	3.3	25	1.8
Federal	13	2.1	6	1.7	12	3.4	0	0.0	31	2.2
Total	37	5.9	14	3.9	26	7.4	4	6.7	81	5.8
Industrial research										
Dairy	3	0.5	5	1.4	6	1.7	0	0.0	14	1.0
Other	4	0.6	5	1.4	1	0.3	0	0.0	10	0.7
Total	7	1.1	10	2.8	7	2.0	0	0.0	24	1.7
Dairy associations	0	0.0	3	0.8	1	0.3	0	0.0	4	0.3
Total	0	0.0	3	0.8	1	0.3	0	0.0	4	0.3
Total	475	75.5	255	70.8	258	73.6	47	78.3	1,035	73.8
	Not related to dairy industry									
U. S. Armed Forces	31	4.9	29	8.1	37	10.5	5	8.3	102	7.3
Commercial enterprises	24	3.8	18	5.0	13	3.7	1	1.7	56	4.0
Professional	49	7.8	25	6.9	15	4.3	0	0.0	89	6.4
Foods	16	2.5	0	0.0	1	0.3	3	5.0	20	1.4
Total	120	19.0	72	20.0	66	18.8	9	15.0	267	19.1
	Address unknown, and deceased									
Unknown	18	2.9	25	6.9	24	6.8	4	6.7	71	5.1
Deceased	17	2.7	8	2.2	3	0.9	0	0.0	28	2.0
Total	35	5.6	33	9.1	27	7.7	4	6.7	99	7.1
Grand total	630	100.1	360	99.9	351	100.1	60	100.1	1,401	100.0

[a] *Central:* Ill., Iowa, Kan., Mich., Minn., Mo., Neb., Ohio, Ontario, Purdue, S. D., and Wis. *Eastern:* Conn., Cornell, Md., Mass., N. H., Pa., R. I., Rutgers, Vt., and W. Va. *Southern:* Ala., Ark., Clemson, Fla., Ga., Ky., La., Miss., N. C., Okla., Tenn., Texas (A & M and Tech.), and Va. *Western:* Calif. (Davis and Poly.), Colo., N. Mex., Ore., Utah, Wash., and Idaho.

mately one-third of the dairy industry graduates. The relatively large numbers of owners and managers are gratifying.

The field of education took about one contestant in seven. Sales took about one in eleven. Departments of agriculture and public health took one in every 17.

About 19% of the former contestants are now employed outside the dairy industry. Of this group, the United States Armed Forces took the largest number, approximately one-third. This is not surprising, since defense services are required and because some, having served in World War II or the Korean War, continued with the Service as a vocation.

Considering that the study involved a 27-yr. period, which included two wars, the number deceased is not surprising. However, those of whom whereabouts are unknown seem unnecessarily large. Admittedly, records of some departments were not as complete as they might have been.

b) Specific jobs within, or allied to, the dairy industry. The various jobs within the dairy industry held by former dairy products–judging contestants seemingly are in keeping with the numerous interests and duties attached to that great industry. The jobs are many and varied, including plant owners, managers, superintendents, foremen, sales, auditors, directors, buyers, fieldmen, technicians, and related work.

To compile a list of specific jobs in or allied to the dairy industry in which 1,035 graduates are employed is next to impossible. Also, such a list would seem to be too extensive and burdensome. Nonetheless, a classification of the major jobs gives perspective to employment possibilities. Such a classification has been attempted briefly (Table 1).

c) Vocations not allied to dairy manufacturing. Jobs outside of dairying held by the contestants are so diversified as to stagger the imagination. These jobs were to be found in many businesses, such as:

Airlines	Limestone
Appliance	Lumber
Banking	Manufacturing
Brewing	Meat packing
Brush manufacturing	Mercantile
Business machines	Motors
Cement	Paper
Coal	Petroleum
Drugs	Publishing
Electric utilities	Real estate
Elevators (grain)	Restaurant
Fertilizer	Seed
Fish	Soup
Fruit	Steel
Furniture	Supermarkets
Hardware	Tobacco
Insurance	

d) Professions. Many former contestants have gone into several of the professions. Among those professions noted were:

Armed services	Lawyer
Banking	Librarian
Baseball	Ministry
Clerk of Court	Police
Dentistry	Postmaster
Domestic	Radio engineer
Fireman	Tax administrator
Foreign Assignee	

Discussion

Analysis of vocations of graduates in dairy industry throughout the United States would seem to be helpful in planning curricula and in placing emphasis on teaching. No such wide-scale analysis of jobs of dairy manufacturing graduates is available. Hence, the study reported herein was undertaken.

Since 467 teams of three men each, a total of 1,401 individuals, competed in the Collegiate Students' International Contest in the Judging of Dairy Products from 1930 to 1956, inclusive, an opportunity was afforded for such a study. Presumably, these contestants, by virtue of their interest in making the team of dairy products judges, were keenly interested in the dairy manufacturing curriculum, and graduated in that field. Obviously, some of them may not have taken their degree in dairy manufacturing. Nonetheless, all were used in the study.

The data presented herein might be interpreted two ways, depending upon the objectives and viewpoints. That approximately three-fourths of the dairy industry graduates remain with the industry for which they were trained would seem to be highly gratifying. On the other hand, some critics, noting that one-fifth of the graduates were employed in other fields of endeavor, might point with alarm or show deep concern over the situation. As a matter of fact, from the educator's viewpoint, the yield of trainees devoted to the industry seems remarkably high. It is doubtful if, in normal times, such a high percentage retention of graduates within the field of their specific training is experienced even by such training centers as the United States Services academies of West Point and Annapolis.

One must be reminded that specific training can never be a complete loss to any industry. So it is with the dairy industry graduates now in other professions. Unquestionably, a paradox does exist among those not presently engaged in dairy industry work. While their pay may come from fields of work unrelated to dairying, it is believed that the majority of them are among the best ambassadors for quality dairy products that the dairy industry ever had. It is inconceivable that a dairy products–judging contestant, trained to sense the various qualities of butter, cheese, milk, and ice cream, could use those products regularly without sensing their qualities and demanding only the best. Surely, they would

be the first to call attention to existing defects in the products. Limited shifts in vocations, even among dairy industry graduates, would seem to be a healthy situation.

Dairy departments take just pride that most men trained in dairy manufactures handle a wide variety of positions well. Naturally, some degree of embarrassment is felt when one of their trained men seeks employment outside the industry. Professors, probably selfishly, like to believe that loyalty of graduates to their training is sufficient to keep them lifelong within that field. Thus, quitting the industry is sometimes needlessly embarrassing both to the graduate and to his professors. Perhaps both overlooked the real purpose of a college education, that is, to fit a person well for a reasonably pleasant, useful, remunerative, full life-work, regardless of its nature.

Success comes, in part, by working in that area which one likes best. In fact, pleasure in one's work is part compensation. No one knows the satisfactions and monotonies of a job better than the man working on a particular job. So, the college graduate trained in dairy industry or elsewhere should seek employment best suited to him, regardless of any possible straining of student-professor relationships. Rather, this fine, near-symbiotic association between student and teacher should be nourished and maintained throughout the years, regardless of the graduate's final work.

Summary

The present employment status of 1,401 contestants in the Collegiate Students' International Contest in the Judging of Dairy Products from 1930 to 1956, inclusive, was studied.

Approximately three-fourths of the contestants are presently employed within, or allied to, the dairy industry. Nearly one-third of them are owners, managers, superintendents, foremen, buyers, auditors, or directors of dairy plants.

Education, sales, farming, field quality control, laboratory, civil service, and industrial research took 14, 9, 7, 3, 2, 6, and 2%, respectively.

Of the 267, or 19%, employed outside and not related to the dairy industry, the United States Armed Forces took 102, or nearly one-third of them. A wide scope of professions including ministry, dentistry, law, and banking was represented.

Slightly over 7% had unknown addresses or were deceased.

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SURVEY OF DAIRY PRODUCTS–JUDGING CONTESTANTS. II. WHAT SALARIES DO DAIRY PRODUCTS–JUDGING CONTESTANTS GET AFTER GRADUATION?

Committee on Judging Dairy Products, A.D.S.A.

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Decreasing numbers of dairy industry graduates at many agricultural colleges cause administrators no little concern as to the reason for such a trend. Some questions confront them. Is the dairy industry graduate being paid wages commensurate with his training? Are college-trained employees hampered by slow promotion in the dairy industry? Has the dairy industry employer properly evaluated the effects of inflation when hiring trained personnel? Do demands of organized labor work to the salary disadvantage of the college graduate? These questions are asked at a time when the industry needs adequately trained men. It is inconceivable that, in an era of automation, well-paying opportunities for gain-

[1] Deceased.

ful employment in such a great industry are nonexistent.

To get first-hand, confidential information from dairy industry graduates concerning their vocational relationships with the industry would seem to be eminently worthwhile. The complete listing of all dairy products-judging contestants in the Collegiate Students' International Contest in the Judging of Dairy Products from 1930 to 1956, inclusive, furnished an opportunity for such a study. Accordingly, a questionnaire was sent to those for whom addresses were known. An analysis of the responses to this questionnaire furnishes the basis for the study reported herein.

Scope of Study and Procedure

In a previous classification and study (Babcock *et al.*, 1958),[1] the present vocational status of the 1,401 contestants in the Collegiate Students' International Contest in the Judging of Dairy Products from 1930 to 1956, inclusive, was obtained. Presumably, almost all of the contestants had majored in dairy manufacturing. Certainly, their intensive training in judging dairy products widened their horizons in respect to the dairy industry.

Confidential information as to their salaries, and likes and dislikes of dairy industry employment, was sought in a two-page questionnaire, returnable in a plain, noncoded, sealed ballot envelope to be inserted in a return-addressed envelope. An accompanying letter explained the purpose of the questionnaire. The point was emphasized that personal data regarding employment were desired as statistics only, and were not to be associated in any way with a particular contestant. Questionnaires were mailed to 1,037 contestants. Some letters were returned because of incorrect addresses. These were forwarded to the various departments from which the contestant graduated, for correction and remailing.

Results

For the most part, the response to the questionnaire was immediate, reflecting more-than-passive interest in the study. Indeed, the spirit manifested in completing the questionnaire, even to the extent, in many instances, of amplifying the information by personal letters, showed a loyalty to dairy products-judging interests not generally anticipated. Up to the time of compilation of results, 569 questionnaires were returned, a yield of 55%.

Of those responding to the questionnaire, 70% were in dairy work (only 30% no longer with the industry). Thus, the respondents represented about the same ratio of nondairy to dairy employment as found in the previous study (Babcock *et al.*, 1958) *(1)*. Based on this high correlation in percentage of nature of employment, as shown by the two groups, the sampling would appear to be highly applicable to all of the contestants. Therefore, the analysis as given was based on the 55% answering the questionnaire. It is believed that had each contestant answered, the results would have been the same.

Salaries of dairy contestants after graduation. The ranges in salary of 569 contestants, grouped according to the years out of college, are presented in Table 1. Only 14, or 2.5%, failed to give any indication of their salary. The remaining 555 indicated their actual salary, range of salary, and/or total salaries, including perquisites. Tabulation of the data brought out some interesting distributions.

Seventy-one per cent of those out of college less than 5 yr. were getting less than $6,000 per year. This percentage declined rapidly with increasing years' experience. Nearly two-fifths,

TABLE 1

Salary groupings of 569 dairy products-judging contestants, by 5-yr. periods after graduation

Range in salary	Distribution of all contestants who were out of college													
	Less than 5 yr.		6–10 yr.		11–15 yr.		16–20 yr.		21–25 yr.		More than 25 yr.		Total	
($)	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
<6,000	82	71.3	42	30.4	2	13.3	14	11.4	12	10.3	8	16.7	160	28.1
6,000 to 8,000	30	26.1	53	38.4	4	26.7	23	18.9	33	28.2	3	6.2	146	25.7
8,000 to 10,000	3	2.6	31	22.5	3	20.0	26	21.3	30	25.6	15	31.3	108	19.0
10,000 to 12,000	....	.....	9	6.5	2	13.3	19	15.6	15	12.8	6	12.5	51	9.0
Over 12,000	....	.....	3	2.2	4	26.7	40	32.8	27	23.1	16	33.3	90	15.8
No salary indicated	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	22	2.5
Totals	115	100.0	138	100.0	15	100.0	122	100.0	117	100.0	48	100.0	569	100.1

38 %, of those out of school 6–10 yr. moved up into the $6,000 to $8,000 bracket. By the time they were out of school 15–20 yr., nearly one-third of the contestants were beyond the $12,000 range. Unfortunately, limited contestants (and hence data) were to be had in the 11- to 15-yr. zone. This grouping represented the 1942- to 1946-graduation period, when contests were not held, due to World War II. Hence, there were few contestant graduates in this out-of-college group.

Specific salaries of contestant graduates. Two hundred and three respondents gave their specific salaries in addition to checking their salary ranges. Another 185 gave the dollar yearly value of the extras, or perquisites, such as products, car, gasoline, housing, and so on. These salary data are classified in Table 2, by years out of college and by salary range. The weighted average salary of 66 graduates out of college less than 5 yr. was $5,120. In addition, the perquisites of 47 of this group had a weighted average of $1,372. While the value of the perquisites changed little with increasing years out of college, ranging from an average low of $1,149 to an average high of

TABLE 2

Average salaries and perquisites of 233 dairy products–judging contestants who gave their specific salaries, by 5-yr. periods after graduation

Contestant out of college	General salary range	Average salaries and perquisites of those giving specific figures					
		Salary			Perquisite		
(yr.)	*($1,000)*	*(No.)*	*(%)*	*($)*	*(No.)*	*(%)*	*($)*
Less than 6	<6	53	22.75	4,664	31	16.75	1,120
	6–8	11	4.72	6,779	14	7.57	2,039
	8–10	2	0.86	8,100	2	1.08	600
	10–12	....	.....	.....	....	.....	.....
	>12	....	.....	.....	....	.....	.....
Total and weighted average		66	28.33	5,120	47	25.41	1,372
6–10	<6	25	10.73	5,078	15	8.11	730
	6–8	24	10.30	6,984	23	12.43	1,218
	8–10	11	4.72	9,187	11	5.94	1,213
	10–12	2	0.86	11,500	6	3.24	950
	>12	1	0.43	16,500	2	1.08	3,750
Total and weighted average		63	27.04	6,907	57	30.81	1,149
11–15	<6	1	0.43	5,364	....	.....	.....
	6–8	1	0.43	7,800	2	1.08	1,000
	8–10	....	.....	.....	....	.....	.....
	10–12	1	0.43	10,500	....	.....	.....
	>12	1	0.43	13,000	2	1.08	1,500
Total and weighted average		4	1.71	9,166	4	2.16	1,625
16–20	<6	4	1.71	5,729	4	2.16	1,575
	6–8	7	2.57	7,080	5	2.70	960
	8–10	8	3.43	9,243	7	3.90	822
	10–12	5	2.15	11,650	3	1.62	2,000
	>12	12	5.15	19,588	12	6.48	1,620
Total and weighted average		36	15.45	12,204	31	16.76	1,364
21–25	<6	7	3.04	5,431	5	2.70	1,170
	6–8	12	5.15	7,153	7	3.78	542
	8–10	12	5.15	9,287	12	6.48	1,254
	10–12	4	0.71	10,022	3	1.62	1,600
	>12	10	4.29	16,900	8	4.32	1,562
Total and weighted average		45	19.31	9,875	35	18.92	1,200
Over 25	<6	6	2.57	5,283	....	.....	.....
	6–8	1	0.43	7,100	1	0.54	500
	8–10	3	1.28	9,420	2	1.08	350
	10–12	2	0.86	10,500	1	0.54	500
	>12	7	2.57	21,360	7	3.78	2,171
Total and weighted average		19	8.15	12,504	11	5.94	1,536
		233	99.99	8,288	185	100.00	1,285

$1,625, the average salary increased to figures beyond $12,500 per year by the time the contestants had been out of college 21 to 25 years.

Wide ranges in salaries were noted within groups out of college a definite period of time. Twelve, ten, and seven contestants out of school 16+, 21+, and 25+ yr. had average salaries of $19,588, $16,900, and $21,300, respectively. The highest individual salaries reported were $25,100, $26,000, $27,000, and $41,000. Likewise, the perquisite values ranged from nothing to as high as $5,000 a year. Among the highest perquisites reported were $3,000, $3,500, $4,200, and $5,000.

Salaries of dairy versus nondairy employees. Difficulty was encountered in obtaining a true, over-all salary index of those currently employed within and without the dairy industry. However, a considerable number from each group did specify their actual salaries. From these reports, the averages were obtained. The data are presented in Table 3.

Regardless of the industry in which employed, graduates reached the highest average salaries when they had been out of college 16–20 yr. However, the highest salary reported, $41,000, was obtained by a graduate not in dairying but in the food industry, who had been out of college at least 25 yr.

From the data reported, graduates remaining in the dairy industry received slightly higher average salaries within the first 5 yr. than those not employed in the dairy industry. This slight salary advantage seemed to be maintained up to 20 yr. After that period, data indicate that nondairy industry–employed graduates received the higher average salaries. The over-all weighted average salary was in favor of those not working in the dairy industry.

Salaries of graduates in specific areas of dairy employment. Data on salaries of former contestants now specifically employed in dairies, public service, and service to dairies are presented in Tables 4, 5, and 6, respectively. A study of the data in these tables is enlightening. Data indicate that the plant owner takes a lower salary than the plant manager. Size of plants involved and failure to include capital gains in the owner salaries reported might account in part for the higher average salary of the managers over that of the plant owners. Plant superintendents received, on the average, a slightly higher salary than assistant managers. Aside from management, sales and advertising positions commanded a higher average salary than any other position connected with the business. Student trainees and relief men, out of college the shortest time, received the lowest average salaries of any group.

Years of experience usually is a factor closely associated with salary of plant employees. Data in Table 4 show that the owners, managers, and sales groups had been out of college an average of 18.7, 15.6, and 14.6 yr., respectively. The respective average salaries of these groups were $9,830, $11,902, and $10,167.

The salary scale for those employed in public service, such as in education and in city, state, and federal employment, is somewhat narrower, with slightly lower averages, than those in the dairy processing and selling industry. The average salary of those in the educational field seems to be directly related to the years out of college which, in turn, is reflected in the professional rank attained. Average salaries reported for dairy services in state, federal, and city areas were $7,225, $7,168, and $6,240, respectively.

Many dairy industry graduates enter fields serving the dairy processing industry, such as jobbers, suppliers, sales engineers, and so on. Without these services, dairy processing would be seriously handicapped. Not only would salaries alone seem to be commensurate with the training and salary, but the perquisites appear to be generous. The average yearly income, salary and income, of 35 men out of college an average of 13.3 yr. was $10,030, (Table 6). That for 18 laboratory owners, chemists, research directors, and/or consultants, out of college 16.8 yr., was $9,840.

TABLE 3

Salaries of dairy versus nondairy employees

Years out of college	Average specific salaries (including perquisites when given) of those contestants working								
	In the dairy industry			Outside the dairy industry			Combined		
(yr.)	*(No.)*	*(%)*	*($)*	*(No.)*	*(%)*	*($)*	*(No.)*	*(%)*	*($)*
<5	47	27.6	5,573	19	30.6	5,183	66	28.4	5,461
6–10	52	30.6	7,293	10	16.1	7,482	62	26.7	7,324
11–15	3	1.8	11,267	1	1.6	5,364	4	1.7	9,791
16–20	23	13.5	13,132	13	21.0	11,407	36	15.5	12,509
21–25	36	21.2	9,950	9	14.5	10,722	45	19.4	10,114
>25	9	5.3	9,078	10	16.1	13,378	19	8.2	11,341
Total and weighted average	170	100.0	8,334	62	99.9	8,995	232	99.9	8,511

TABLE 4

Salaries of graduates doing specific dairy work

Type of work	Distribution and salaries of dairy graduates			
	No.	Years out of college	Salary ($) Range	Salary ($) Average
Owner or co-owner of a dairy plant (including ice cream and restaurant, milk, cheese, soft ice cream, etc.)	19	18.7	6,000–27,000	9,830
Manager of dairy plant (milk, cheese, ice cream, butter, recombined milk)	61	15.6	5,500–22,000	11,902
Assistant manager	10	10.6	5,160–12,500	8,418
Plant superintendent	56	12.5	5,100–14,000	8,751
Assistant superintendent, production manager, and/or foreman	30	9.8	4,383–11,000	6,605
Laboratory: director, technician, chemist	12	11.4	4,160–11,000	6,660
Procurement, quality control	13	12.8	5,304–11,750	7,046
Sales and advertising	9	14.6	6,000–13,000	10,167
Relief men and student trainees	6	5.5	4,500– 6,000	5,423
Miscellaneous (public relations, comptroller)	3	7.0	5,200–10,000	6,973
Fieldman	10	13.1	3,600– 8,500	6,311
Total and weighted average	229		3,600–27,000	9,016

Several other jobs related to the dairy processing industry and not included in the foregoing tables were reported. Most of them were closely associated with dairy farm operations, as managers and owners. Difficulty was encountered in presenting a true picture of the salaries connected with these jobs, for the reason that few of them included the perquisites, such as house, car, gas, farm products, and so on, usually incident to rural living. Twenty-four engaged in the farming enterprise reported an average salary of $6,611. Seven of them reported perquisites averaging $1,471. The average income of the 24, including the seven reporting perquisites, was $7,039.

Salaries of graduates in specific nondairy employment. The specific nondairy jobs presently held by former contestants are so varied as to defy classification. However, a glance at the occupations suggests the following possible general grouping: *a)* public service (federal and state); *b)* education; *c)* farming; *d)* military service; *e)* professional, and *f)* miscellaneous. The miscellaneous group contains the largest number and widest variety of jobs. Among them are to be found examples

TABLE 5

Salaries of graduates in university, city, state, and/or government work

Area of employment	Rank and/or nature of work	Distribution and salaries of graduates			
		No.	Years out of college	Salary ($) Range	Salary ($) Average
University teaching, research, and administration	Professor [a]	13	20.1	6,000–12,000	9,935
	Associate professor	11	12.0	5,520–11,000	8,138
	Assistant professor	9	10.7	6,700– 7,652	7,130
	Instructor	3	6.0	4,800– 6,800	5,900
	Research assistant	5	<5.0	1,860– 6,000	3,672
City	Sanitarian	2	13.0	6,000– 6,480	6,240
State	Director, inspector, bacteriologist	10	14.4	5,400–11,100	7,225
Federal	Director, inspector, technician, marketing specialist, grader, etc.	12	16.0	5,000– 9,850	7,168

[a] Including several heads of departments.

TABLE 6

Salaries of graduates in occupations serving dairy industry but not employed in a processing plant

Type of work	Distribution and salaries of graduates			
	No.	Years out of college	Salary ($)	
			Range	Average
Salesmen, representative of suppliers, engineers, jobbers, etc.	35	13.3	5,200–19,100	10,030
Chemist, research director, owner dairy laboratory, consultant, etc.	18	16.8	5,400–15,000	9,840

of almost all the jobs incident to daily life of modern civilization. That they are handling these jobs well is shown by their range and average salaries. Eighty-nine of them averaged $9,940, including perquisites. In this group also were to be found such yearly salaries as $21,000, $26,000, and $41,000.

Discussion

A study of the returned questionnaires pertaining to salary alone seems to have made the effort highly worthwhile. Individually, and as a group, the former contestants appear to have done very well financially. The wide variety of jobs currently held by them indicates that that same judgment used in judging dairy products was exercised in selecting a vocation best suited to their liking and qualification.

University administrators and guiding professors often are perplexed as to what constitutes the best curriculum for the different students' interests. The trend of thinking today in many agricultural colleges is to minimize and discourage collegiate courses lacking in basic principles. Such courses as Judging Dairy Products at some institutions either are not offered or, if listed, are merely tolerated without pride. Repeatedly, former contestants, whether now gainfully employed in the dairy industry or in some other enterprise, testified to the benefits derived from their experience in participating in the Collegiate Students' International Contest in the Judging of Dairy Products, an extracurriculur activity associated with collegiate studies. They emphasized the encouragement and the inspiration which their judging experience gave them.

Back of the student-contest opportunity to intermingle with other college students in friendly rivalry were the weeks of intensive training under some professor. Perhaps it is not too presumptuous to say that the influence of these professors is reflected in the present financial success of these contestants.

Unhesitatingly, many former contestants stated that judging dairy products was "the best course I had in college." Learning the different qualities of butter, cheese, milk, and ice cream was the primary objective. Unquestionably and unconsciously, in doing so, they came to know intimately a coach who believed in and put faith in their ability. This in itself must have had a positive influence on their success after graduation.

Summary and Conclusions

Salary and type-of-employment distribution studies were made of 569 contestants who participated in the Collegiate Students' International Contest in the Judging of Dairy Products from 1930 to 1956, inclusive. These respondents represented 55% of the 1,037 former contestants to whom ballot-envelope-returnable questionnaires were sent. Based upon a previous analysis of vocations involved, which coincided remarkably well with the jobs of those responding, this sampling appears to be statistically representative of the mass.

Seventy-one per cent of the graduates out of college less than 5 yr. were in the salary bracket under $6,000. Nearly one-third of those out of college 15–20 yr. were beyond the $12,000 level.

One hundred and seventy dairy industry–related workers, reporting their specific salaries, averaged $8,334; whereas, 62 nondairy-employed graduates averaged $8,995. Eighty-nine nondairy employees, indicating their general and/or specific salaries, with perquisites, averaged $9,940 yearly. Of this group, top salaries reported were $21,000, $26,000, and $41,000. The top salaries reported by those presently employed in the dairy industry were $25,100 and $27,000.

Rating of jobs in dairy plants according to average salaries was as follows:

Manager, sales, owner, superintendent, assistant manager, procurement and quality control, laboratory, assistant superintendent, fieldman, and student trainees. Plant owners reported the widest range in salaries; student trainees the narrowest.

Average salaries of graduates presently engaged in education and public service were

markedly lower than those of graduates connected with dairy plants and/or in nondairy and private enterprise. Average salaries of educators were directly related to the years out of college.

As a group, graduates attain the highest average salary from 16 to 20 yr. following graduation.

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(1) Babcock, C. J., Dowd, L. R., Downs, P. A., Thomas, E. L., Warren, F. G., Willingham, J. J., and Trout, G. M. Survey of dairy products-judging contestants. II. What salaries do dairy products-judging contestants get after graduation? *J. Dairy Sci.*, **45**: 1826. 1958.

THE 3-A SANITARY STANDARDS COMMITTEES—THEIR WORK AND CONTRIBUTIONS

J. R. Brunner[1]

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Since the publication of the first Sanitary Standard in May of 1947, the 3-A Sanitary Standards Committees have been engaged in one of the most important assignments ever given an industry group. From their deliberations have come the recommended standards, specifying the construction of dairy processing equipment. As a practical result, equipment manufacturers, sanitarians, and public health officials will be able to exert a common effort toward providing the consumer with wholesome dairy products.

The membership of A.D.S.A., especially those members in academic work, should know the composition of these committees and how they function. Briefly, the committees are composed of representatives from *a)* the Committee on Sanitary Procedures (CSP), International Association of Milk & Food Sanitarians, Inc., *b)* Milk and Food Program, U. S. Public Health Service, and *c)* the Sanitary Standards Subcommittee (SSS), Dairy Industry Committee. The over-all objective is to formulate and encourage acceptance, through the mutual cooperation of these agencies, representing the sanitarian, manufacturer, and user groups, of 3-A Sanitary Standards specifying sanitary criteria for equipment used by the industry.

The basic mechanism required to formulate a standard commences with the presentation of an initial draft, formulated by a qualified task group composed of representation from the Dairy Industries Supply Association. From this beginning, a standard is developed to the point where not only its basic concept but the most elementary details are acceptable to all the constituent committees. The adoption of the standard by the 3-A Sanitary Standards Committees is signified by the subsequent publication of the standard in the *Journal of Milk and Food Technology*. The procedure followed to produce a standard is presented graphically in Figure 1. To date, some 28 standards, revisions, and/or supplements have been published; 12 active pending standards or supplements remain on the 3-A agenda. Others will be added as the need becomes apparent. The entire set of published standards can be obtained by sending $2 to Mr. H. L. Thomasson, Box 434, Shelbyville, Indiana.

In the role as A.D.S.A. observer to the 3-A Sanitary Standards Committees during the past year, the writer was impressed with the performance of this group—the thoroughness with which every detail of a standard was considered before being passed on to the next group for consideration and approval. Writing a standard suitable for current conditions, but not so restrictive as to restrain future developments in equipment and technique, presents a real challenge. The committees are alert to changing conditions within the industry which warrant a reconsideration of existing standards. An established procedure for amendment or supplementation is followed when modification is indicated.

It was gratifying to learn that many of the members of the various 3-A committees are active members of A.D.S.A. All of these people are contributing their time and effort to the project, motivated principally by the realization of the importance of this industry-wide project. Their efforts contribute significantly to the over-all strength of the dairy industry, present and future. These committees face an ever-increasing agenda as new processing equipment and techniques are introduced. The manufacturers of dairy equipment, as well as the sanitarians and processors, are very much aware of the importance and scope of this work. But are the instructors of dairy and food technology as familiar with the 3-A Sanitary Standards as they should be? Are there sets of the published standards in our libraries and reading-rooms, for staff and

[1] A.D.S.A. observer-representative to 3-A Sanitary Standards Committees for 1957-58.

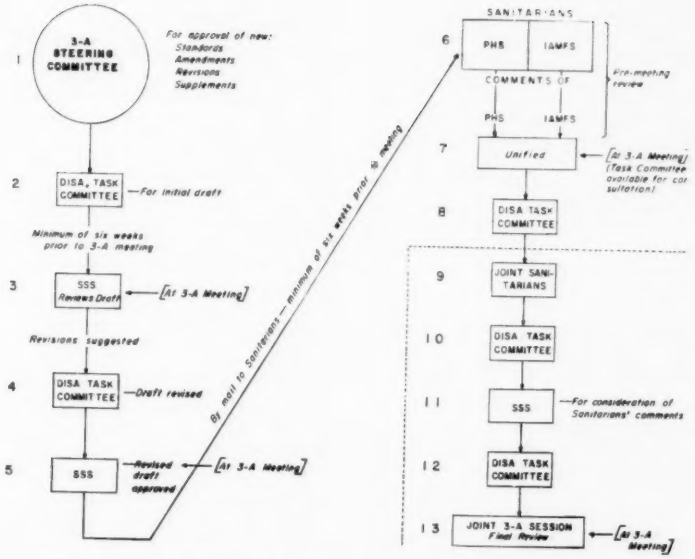

FIG. 1. Procedure followed by the 3-A Sanitary Standards Committees for the approval of new standards, amendments, revisions, and supplements.

students to study? How much time is spent acquainting students with the work of 3-A Sanitary Standards Committees?

Possibly, these questions and others should be considered. If deficiencies exist, then steps should be taken to strengthen our programs. After all, many of our graduates take positions in which a knowledge of sanitation and the 3-A Sanitary Standards is essential. Then, too, it would seem that instructors should have an active interest in the proceedings of the 3-A committees. Realizing this obligation, and with an invitation from the 3-A committees, recent presidents of A.D.S.A. have named academic observer-representatives to sit with the 3-A Sanitary Standards Committees. A report from these observers is submitted to the president prior to the annual meetings. The reading or distribution of this report at the annual business meeting would seem to serve a worthwhile purpose. Furthermore, it is possible for the membership of A.D.S.A., especially those in the teaching profession, to submit recommendations to the 3-A committees through these 3-A observers. The 3-A committees are especially interested in learning of research results from investigators who are working on equipment design and cleanability. To this end, the A.D.S.A. can contribute significantly to the important work of these committees.

ABSTRACTS OF PAPERS PRESENTED AT THE EASTERN DIVISION MEETING

SPRINGFIELD, MASSACHUSETTS, SEPTEMBER 15, 1958

Some interrelationships between vitamin E and vitamin A in Holstein calves. MARTHA W. DICKS, J. E. ROUSSEAU, JR., H. D. EATON, R. TEICHMAN, A. P. GRIFO, JR., AND H. A. KEMMERER, JR., Storrs (Conn.) Agricultural Experiment Station, Storrs.

Thirty-six Holstein male calves, 65 days of age and partially depleted of their tocopherol and vitamin A stores, were fed in addition to a basal ration low in tocopherol and vitamin A, one of three levels of tocopherol acetate, 1, 5, or 25 mg. of free tocopherol per pound live weight per day, and one of three levels of vitamin A palmitate, 10, 100, or 1,000 γ as vitamin A alcohol for a 4-wk. period. Upon completion of a 28-day to-

copherol–vitamin A supplementation period, one-half of the calves were slaughtered and the other 18 calves were maintained on only the basal ration until plasma vitamin A decreased to 4.0 γ/100 ml. Based on plasma and liver vitamin A concentrations at the termination of supplementation and on vitamin A depletion time, tocopherol caused increases in utilization of vitamin A at the highest intake of vitamin A, decreases at the lowest intake of vitamin A, and inappreciable change at the middle intake. Across all levels of tocopherol intake, the tocopherol concentration of the tissues decreased with increasing intake of vitamin A. This decrease was greater between the 10 and 100 γ intake of vitamin A than between the 100 and 1,000 γ intake. These results indicated a need to establish levels or ratios of intakes for both vitamins A and E.

Sensitivity of various tissues of Holstein calves to tocopherol intake. A. P. GRIFO, JR., H. D. EATON, AND J. E. ROUSSEAU, JR., Storrs (Conn.) Agricultural Experiment Station, Storrs, and L. A. Moore, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Data obtained at the termination of three experiments on the total tocopherol concentrations of liver, heart, trapezius muscle, and perinephric fat of Holstein male calves, receiving graded levels of tocopherol added to a basal ration in daily amounts ranging from 0.125 to 25.000 mg. per pound of live weight, and for periods ranging from 4 to 12 wk. duration, were studied for the purpose of determining the relative sensitivity of these tissues in reflecting tocopherol intake. Using the sensitivity concept of Mandel and Stiehler (1954), which in this study was the ratio of the rate of linear change of the logarithmic (Base 10) tissue concentration of tocopherol on the logarithmic intake of tocopherol to the standard deviation of the logarithmic tissue tocopherol concentration, it was found that the sensitivity was in the following descending order: heart, liver, perinephric fat, and trapezius muscle. The first two named tissues were found to be approximately twice as sensitive to tocopherol intake as the latter two. Expressing tocopherol concentration on a per gram of lipid basis resulted in inappreciable change in sensitivity, as compared to a wet basis as used above.

Effect of exogenous hormones on the reproductive processes of the cycling dairy heifer. J. S. GREENSTEIN, R. W. MURRAY, AND R. C. FOLEY, Massachusetts State University, Amherst.

Hormone preparations of progesterone, estradiol, human chorionic gonadotrophin (HCG), and pregnant mare serum (PMS) were administered subcutaneously and separately to reproductively normal, cycling animals at several dosage levels and at various stages of the estrous cycle, in attempts to modify reproductive processes. Their effects were studied in the living animals by rectal palpation, by observations on cycle length, and by daily basal temperature determinations. Along with appropriate controls, these animals were later slaughtered at predetermined stages of the estrous cycle and histological studies were made on the corpora lutea and endometria.

Preliminary findings indicate that 50 mg. of progesterone administered daily beginning on Day 12 post-estrus inhibits heat and ovulation. Suppression of follicular development and early regression of the corpus luteum result from daily treatment with 1–2 mg. of estradiol from Day 2 to Day 12. Daily injections of 1,000 I.U. of HCG, beginning on Day 12, induce development of cystic follicles accompanied by prolonged cycle length. One thousand International Units of PMS administered on Day 6 produces prolonged cycle length and inhibition of ovulation, whereas superovulation is induced when administered on Day 18.

Daily vaginal temperature determinations proved unreliable as an index of individual cyclic behavior patterns. No thermogenic response to exogenous progesterone was noted in intact heifers.

Effect of stage of lactation and pregnancy on the solids-not-fat content of cow's milk. C. J. WILCOX, K. O. PFAU, R. E. MATHER, AND J. W. BARTLETT, New Jersey Agricultural Experiment Station, Sussex, N. J.

Monthly milk samples from the Station's Holstein and Guernsey herds were tested for solids-not-fat (SNF) percentage from June, 1953, to February, 1958. From a total of 4,772 samples tested by methods involving specific gravity (Watson Pattern Lactometer) and fat percentage (Babcock), SNF averaged 8.47% for Holsteins and 8.93% for Guernseys.

After removing the effects of calendar months by appropriate statistical procedures, stage of lactation proved to have a highly significant effect on SNF percentage. A characteristic lactation curve was in evidence. SNF per cent was high shortly after parturition, dropped to a lactation low at 40–60 days, increased slowly to 6 mo., then increased rapidly to the end of the lactation. Grouping of cows according to pregnancy status revealed that much, if not all, of the increase in SNF per cent in the latter stages of lactation was associated with pregnancy. These effects became apparent during the 4th or 5th mo. of gestation. Farrow cows increased little or not at all.

Results agreed well with those of other investigations which were based on SNF determinations by laboratory methods. Use of these field techniques seemed to be adequate for studies of this type.

Effect of nitrogen fertilization and stage of maturity on the nutritive value of bromegrass hay. N. F. Colovos, H. A. Keener, N. K. Peterson, P. T. Blood, and H. A. Davis, New Hampshire Agricultural Experiment Station, Durham.

The nutritive evaluation of brome-grass hay grown under two levels of nitrogen fertilization (50 and 200 lb. per acre) and harvested at three stages of maturity was studied with twin steers. The evaluations were made on the basis of digestible dry matter, digestible protein, digestible energy, and total digestible nutrients.

Stage maturity affected the nutritive value of the hays grown under the two levels of fertilization in all methods. The level of nitrogen fertilization did not affect, significantly, the digestible dry matter, digestible energy, or total digestible nutrients. The digestible protein, however, was significantly higher, 10–15%, in the hays grown at the high nitrogen fertilization.

Influence of vacuum pasteurization upon milk loss. A. C. Smith, L. R. Glazier, D. G. Goslee, and L. R. Dowd, Storrs (Conn.) Agricultural Experiment Station, Storrs.

This study shows increases in percentage of milk loss with increasing degrees of flash-cooling when processing homogenized milk at 172° F. in various adaptations of a Vacu-Therm Unit. Three or four replicates with four different degrees of flash-cooling increments were run on each adaptation. The percentage of milk lost was calculated from Mojonnier total solids determinations on the raw and treated milks. The upward trend in the percentage of milk lost, with increasing degrees of flash-cooling in the various vacuum chambers, was expressed by the following equations: (1) $Y = -0.345 + 0.127X$ (single-vacuum chamber before final heating); (2) $Y_1 = 0.004 + 0.061X$ (single-vacuum chamber before final heating and with a two-inch by four-foot tubular condenser located in the vapor line at a 30° slope and operated with a 3° F. vapor temperature drop); (3) $Y_1 = 0.172 + 0.047X$ [single-vacuum chamber after pasteurization with a condenser as noted in (2)]; (4) $Y_1 = -0.052 + 0.035X$ (double-vacuum chamber with the first chamber before final heating and the second chamber after pasteurization); (5) $Y_1 = -0.084 + 0.034X$ [double-vacuum chamber as noted in (4) with a condenser as noted in (2)]; where Y represents the percentage of milk loss; Y_1 the square root of the percentage of milk loss, and X, the degrees of flash-cooling.

In-place cleaning of vacuum flavor removal equipment. L. R. Glazier, A. C. Smith, and L. R. Dowd, Storrs (Conn.) Agricultural Experiment Station, Storrs.

This study shows that some types of vacuum flavor removal equipment can be successfully cleaned in place. A double Chamber Vacu-Therm unit was cleaned in place for 6 mo. and a procedure system established. Swab counts were run during a 7-wk. period. No hand-cleaning of suspicious-looking areas was done. For best results, it was found necessary to install a spray tube in the top of each chamber. Swab counts at the end of the 7-wk. period compared favorably with those taken during the first week. Visual inspection showed satisfactory cleaning of the vacuum equipment and vapor lines as well as the high-temperature, short-time system.

A study of certain processing procedures on some properties of the half-and-half milk product. F. E. Potter, Massachusetts State University, Amherst.

The half-and-half milk product containing 10% fat was prepared, using either blends of cream and skimmilk or cream and whole milk. The products were pasteurized at 150° F. for 30 min. and homogenized under various conditions. Samples homogenized at less than 2,000 lb. p.s.i. exhibited cream plug formation within seven days of storage at 35° F. Further evidence of inefficient homogenization was obtained by light transmission studies. Single-stage homogenization gave a slightly more viscous product than did double-stage homogenization. In double-stage homogenization, a slightly more viscous product resulted when 300 lb. was used on the second stage, compared to 500 lb. The addition of milk solids-not-fat also increased the viscosity of the product. In using cream from cold milk separation, a slightly more viscous product was obtained, as compared to the use of cream from warm milk separation. When the properly homogenized products were added to hot coffee there were no indications of "feathering" or "oiling-off."

Dairy products research in the U. S. Department of Agriculture. W. I. Patterson, Chief, Dairy Products Laboratory, Eastern Utilization Research and Development Division, U. S. Department of Agriculture, Washington, D. C.

The Eastern Utilization Research and Development Division has an active research program on major dairy products, especially dry whole milk powder, evaporated milk, butter fat, and cheese. Two approaches are used in our studies on these problems. First, further research on milk itself is important to improvements in present milk products. The chemical changes in the components of milk during the various processing treatments which it undergoes during the manufacture of dairy products are believed to be responsi-

ble for the undesirable properties and flavor qualities which may be characteristic of some dairy products. A more thorough knowledge of the chemistry of milk will give clues to ways of preparing better milk products.

The second phase of our program involves the development of practical improvements in presently available products and the discovery of new dairy products, with the goal of increasing consumption of milk. A number of new research developments will be described.

PROGRAM FOR THE 1959 MEETING OF THE SOUTHERN DIVISION

PEABODY HOTEL, MEMPHIS, TENNESSEE

Monday, February 2

8:30—10:45 A.M.

Peabody Hotel, Jacobean Room

J. W. POU, *Chairman*

1. Implications of the Differences Between the Measured Butterfat Value and the Calculated Butterfat Value of a Dairy Herd. R. W. HENNINGSON, Clemson Agricultural College.
2. The Influence of a High Fat Ration upon the Composition of Milk and Milk Fat. I. I. PETERS, R. R. HARRIS, C. A. MULAY, and F. PINKERTON, Texas Agricultural Experiment Station.
3. Effect of Height of Shade on Solar Radiation Receipt Under Louisiana Summer Climatic Conditions. J. E. JOHNSTON, F. T. WRATTEN, F. B. HAMBLIN, and A. GUIDRY, Louisiana State University.
4. Comparison of Green-Chopped Starr Millet and Alfalfa Silage for Lactating Dairy Cows. L. J. BOYD, University of Tennessee.
5. The Effect of Intrauterine Antibiotic Treatment on Low-Fertility Dairy Cows. S. D. MUSGRAVE, W. N. PHILPOT, and E. W. JONES, Oklahoma Agricultural Experiment Station.
6. A Report of the Use of Commercial Cellulase on Various Plant Materials. D. S. RAMSEY, Mississippi State University.
7. Intensity of Estrus in Dairy Cattle in Louisiana. J. G. HALL and C. BRANTON, Louisiana State University.
8. Strontium and Calcium Metabolism in Dairy Cows. R. G. CRAGLE and B. J. DEMOTT, University of Tennessee.

11:00 A.M.—12:30 P.M.

GENERAL SESSION, A.S.A.W.

Peabody Ballroom

2:00—4:15 P.M.

Peabody Hotel, Jacobean Room

T. R. FREEMAN, *Chairman*

9. Use of Zinc Bacitracin as a Silage Preservative—A Preliminary Report. L. L. RUSOFF, C. P. BREIDENSTEIN, W. J. MILSTEAD, and J. E. BERTRAND, Louisiana State University.
10. Fermentation Characteristics of Alfalfa Silages. ANN R. STASCH and M. E. MCCULLOUGH, Georgia Experiment Station.
11. Progress and Problems in Production Testing for SNF Yields of Individual Cows. N. R. THOMPSON, W. K. STONE, and G. C. GRAF, Virginia Polytechnic Institute.
12. Comparison of Coastal and Common Bermuda Hays with Oat and Alfalfa Hays for Milk Production. W. A. KING, Clemson Agricultural College.
13. The Effect of Grain-to-Milk Ratio on the Milk Production of Dairy Cattle. H. D. ELLZEY, Louisiana Southeast Experiment Station.

Special Progress Report from American Dairy Association on "Research on Butterfat in Relation to Current Health Problems." D. H. JACOBSEN.

Tuesday, February 3

8:30—10:00 A.M.

Peabody Hotel, Jacobean Room

J. T. MILES, *Chairman*

"The Dairy Curriculum." Committee Report. H. C. OLSON, I. W. RUPEL, and H. B. HENDERSON, *Chairman.*

"How Do We Fulfill the Demand for Dairy Graduates?" Panel discussion. L. J. BOYD, *leader.*

(1) The Current Demand for Dairy Graduates. H. B. HENDERSON.

(2) How We Encourage High School Students to Attend College and Major in Dairying. T. R. FREEMAN.

(3) How We Attract Future Dairy Majors. G. C. GRAF.

10:00—11:00

Sectional Business Meeting

J. W. POU, *Presiding*

11:00 A.M.—12:30 P.M.

General Session, A.S.A.W.

Peabody Ballroom

2:00—4:00 P.M.

Dairy Manufacturing, Peabody Hotel, Pompeian Room

A. V. MOORE, *Chairman*

14. The Growth of Certain Lipolytic Psychrophilic Organisms in Pasteurized Milk. W. W. OVERCAST and J. D. SKEAN, University of Tennessee.
15. An Evaluation of Milk Quality from the Point of View of the Consumer. CALVIN B. REEVES, Clemson Agricultural College.
16. Preservation of Lactic Cultures. H. C. OLSON, Oklahoma State University.
17. The Effect of Various Factors on the Activity of Frozen Cultures. J. T. CARDWELL, T. J. OUSLEY, and J. H. MARTIN, Mississippi State University.
18. Factors Affecting the Spreadability of Butter—A Preliminary Report. J. B. MICKLE, Oklahoma State University.
19. Rancidity Resulting from Fat Shearing in Raw Milk. A. V. MOORE, A & M College of Texas.

2:00—4:15 P.M.

Dairy Production, Peabody Hotel, Jacobean Room

W. A. KING, *Chairman*

20. Measures of Persistency of Lactation. J. LANE FLETCHER, Agricultural Research Service, USDA.
21. An Economic Analysis of Virginia Dairy Herd Improvement Association Records. WILLIAM S. GRIFFITH and K. E. LOOPE, Virginia Polytechnic Institute.
22. The Effect of Preservatives on the Silo Losses and Feeding Value of Alfalfa Silage. L. R. SISK, M. E. MCCULLOUGH, and O. E. SELL, Georgia Experiment Station.
23. Optimum Replacement Rates for Effective Herd Improvement Through Breeding. I. W. RUPEL, A & M College of Texas.
24. Intrauterine Antibiotic Treatment of Low-Fertility Dairy Cows. W. N. PHILPOT, S. D. MUSGRAVE, and E. W. JONES, Oklahoma State University.
25. Effect of Torula Yeast on the Yield and Composition of Milk. L. J. BUSH and E. R. BEROUSEK, Oklahoma State University.
26. Carotene Requirements of Dairy Cattle. MAGNAR RONNING, E. R. BEROUSEK, WILLIS D. GALLUP, and J. L. GRIFFITH, Oklahoma State University.

6:30 P.M.

Annual Banquet and Honors Program, Peabody Hotel

Wednesday, February 4

8:45—11:00

Peabody Hotel, Jacobean Room

G. C. GRAF, *Chairman*

27. Effect of Air Conditioning and Other Cooling Practices on Physiological Responses, Semen Production, and Fertility of Bulls Under Southern Conditions. T. E. PATRICK, Louisiana State University.
28. Effects of Fiber Content of the Ration on Milk Production and Hot-Weather Discomfort of Producing Dairy Cows. I. W. RUPEL, A & M College of Texas.
29. Comparison of Protein Supplement and Shelled Corn for Dairy Cows on Pasture. H. T. BRYANT and R. E. BLASER, Virginia Polytechnic Institute.
30. In Vivo Studies on the Deamination of Amino Acids by Rumen Microorganisms. C. G. LOOPER and O. T. STALLCUP, University of Arkansas.
31. Free-Choice Supplementation of Corn and Sorghum Silage Rations Fed to Growing Dairy Heifers with a Urea-Alcohol-Molasses Preparation. KENNETH BIERWORTH and O. H. HORTON, University of Arkansas.
32. The Effect of Subnormal Growth of Dairy Heifers upon Their First Lactation. E. W. SWANSON, A. F. MCFEE, L. M. CARPENTER, and S. A. HINTON, University of Tennessee.
33. The Influence of Various Diets on Rumen Development in Dairy Calves. W. C. LOE, O. T. STALLCUP, and H. W. COLVIN, JR., University of Arkansas.

ERRATA

MOORE, W. E. C., AND KING, K. W. Determination of the Intraruminal Distribution of Soluble Nitrogen. *J. Dairy Sci.*, **41:** 1451. 1958.

Last sentence under Results and Discussion should be: Analysis of internal standards consisting of casein, glutamic acid, and ammonium sulfate demonstrated that the recoveries of nitrogen were in excess of 95%.

Corrected Table 2 should be as follows:

TABLE 2

Intraruminal distribution of nitrogen as affected by ration

	Ration					
	Hay		Grain		Grain + Tryptone	
	(γN/ml)	*(% of total N)*	*(γN/ml)*	*(% of total N)*	*(γN/ml)*	*(% of total N)*
I. Total-N	1,156	.....	1,117	.....	1,063	....
A. Particulate-N (1-B)	927	80.2	848	76.0	676	63.5
B. Soluble-N	229	19.8	269	24.1	387	36.4
1. TCA-soluble-N	212	18.3	257	23.0	395	37.1
2. NH_3-N	97	8.4	100	9.0	116	10.9
3. Soluble protein-N (B1)	17	1.5	12	1.1	−7	−0.7
4. Organic NPN (1-2)	115	10.0	157	14.1	279	26.2
% Recovery[a]	.....	100.1	.....	100.2	.....	99.9

[a] Calculated as $\frac{A+2+3+4}{\text{total}} \times 100$.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL HEALTH

285. Lactate composition for treatment of bovine ketosis. J. C. SHAW. U. S. Patent 2,856,326. 8 claims. October 14, 1958. Offic. Gaz., U. S. Patent Office, **735**, 2: 456. 1958.

Ketosis in cows is controlled by feeding a mixture of 15–85% calcium lactate and 85–15% of sodium or ammonium lactate.

R. Whitaker

286. Lactate-lactobionate composition for treatment of bovine ketosis. J. C. SHAW AND J. J. JONAS. U. S. Patent 2,856,327. 12 claims. October 14, 1958. Offic. Gaz., U. S. Patent Office, **735**, 2: 456. 1958.

Ketosis in cows is controlled by feeding a mixture of sodium or ammonium lactobionate, calcium lactate, and sodium or ammonium lactate having a pH of between 5 and 6 in a 10% solution at 25° C. R. Whitaker

BOOK REVIEWS

287. Dairy Handbook and Dictionary. J. H. FRANDSEN, Ed. Published by J. H. Frandsen, Amherst, Massachusetts. 843 pp. 1958.

Nearly 460 pages of this book comprise a dictionary of terms used both in dairy production and in dairy manufacturing. Many of these terms are taken from the fields of chemistry, physics, bacteriology, physiology, genetics, and veterinary medicine. A descriptive approach is used in defining the numerous terms, with some useful details given in many instances. One finds directions for running laboratory tests on dairy products; formulations for milk drinks, ice cream, and various cream preparations; a long list of feeds, including analyses; a list of cattle diseases, with appropriate discussion; description of all types of milk-processing euipment, and a wealth of information on varieties of cheese and cheese terms. There is hardly any branch of the dairy industry which has not received its due consideration.

Some 276 pages are devoted to a Dairy Handbook. It consists of 46 rather brief papers by nearly as many authors. It is again evident that an effort was made to cover every aspect of the dairy industry. The papers deal with feeding and management of the dairy herd, essentials of breeding, sanitation on the farm, milk pasteurization, butter and cheese making, ice cream manufacture, dried by-products of milk, cleaning materials and procedures, maintenance of equipment, and marketing problems. Also included are papers on the role of chemistry and bacteriology in the dairy industry, flavor of milk, nutritive value of milk, control of insects, farm structures, forage crops, and breeds of dairy cattle. For the most part, the papers are aimed at giving an over-all picture of each subject, with only enough detail to maintain clarity of presentation.

An extensive reference section is provided, which consists of tables on milk composition, consumption data, federal standards for dairy products, space requirements for various feeds, calibration of Babcock glassware, dairy organizations and publications, and other useful information.

This book is a convenient and extensive source of information on any branch of the dairy industry. J. Tobias

288. The Metabolism of Lipids. British Medical Bulletin, Vol. 14, No. 3. Edited by G. Popjak. $3.25. September, 1958.

This symposium consists of 12 papers by 20 leading British investigators concerned with various aspects of lipid metabolism.

One of the major problems in the area of lipid metabolism is the etiology of atherosclerosis. The majority of the papers presented are concerned with this multifaceted disease. Bronte-Stewart discussed the effect of dietary fats on the blood lipids. The role of the essential fatty acids in humans was discussed in two other papers, by Sinclair and by James and Lovelock. Other papers discussed the removal of lipids from the blood stream, the hormonal control of circulating lipids, disorders in fat absorption, and fats and blood coagulation.

The basic aspects of lipid metabolism were reviewed under discussions of biosynthesis of fatty acids, biosynthesis of cholesterol, and the absorption and metabolism of sterols.

Finean and Robertson presented a paper on

lipids and the structure of myelin. This was the only paper in the series on the role of lipids as cell structural components.

In his introduction to the symposium, Frazer gave a concise and critical abstract of the papers presented, and pointed out the various areas in lipid metabolism which offer promising fields of discovery to the biochemist, biologist, and chemist. F. A. Kummerow

289. Recommended Methods for the Microbiological Examination of Foods. American Public Health Association, Inc., New York. 212 pp. $4.50. 1958.

This publication is the first attempt to produce some degree of uniformity in the microbiological examination of various foods. It consists of methods used throughout the food industry, and includes information that formerly was not only widely scattered but in some instances virtually unavailable.

The methods outlined represent a vast screening and evaluating of the many possible methods available. Each method is presented simply and completely, so that one unfamiliar with the particular food product might easily follow the procedure. Fourteen general groups of food products are treated in separate chapters. Each chapter usually has an introductory statement about the food, sections on the handling of samples, the applicable microbiological procedures, and the interpretation of results. Three additional chapters deal with general directions, sanitation indexes, and detection and enumeration of food-poisoning types of microorganisms. The culture media, stains, and reagents and indicators mentioned in the text are found in three appendixes. The culture media are arranged in alphabetical order and include the composition and directions for preparation and use. Pertinent references are cited throughout the book and an adequate index to the text material is included.

It would be surprising, and perhaps even unfortunate, if this collection of methods does not receive criticism. Each laboratory dealing with foods has its own routine methods of sampling, culture media, incubation conditions, and interpretation of results, which probably differ at least in part from those listed here. Nevertheless, these Recommended Methods should be greeted with enthusiasm by all those interested in the microbiological examination of foods. L. D. Witter

CHEESE

290. Cheese process and resulting product. D. M. Irvine and W. V. Price. U. S. Patent 2,850,390. 2 claims. September 2, 1958. Offic. Gaz., U. S. Patent Office, **734**, 1: 198. 1958.

High-moisture, quick-curing cheese, suitable for use in the manufacture of cheese spreads, is made in the conventional way up to the time the whey is drained. When at least 75% of the whey is removed, the curd is soaked in from 2 to 4% brine. The mixture is held to allow the starter organisms to develop acid to a pH of 5.3–5.7. More salt is added to make a brine concentration of from 4 to 6%. The curd-in-brine mixture is heated from 110 to 130° F. The salty whey is drained and the curd cured. R. Whitaker

291. Cheese curd-fusing machine. J. Czulak and L. A. Hammond. U. S. Patent 2,851,776. 2 claims. September 16, 1958. Offic. Gaz., U. S. Patent Office, **734**, 3: 570. 1958.

Curd with some whey is filled into a chamber and compressed by means of a piston, to squeeze out free whey and partially knit the curd particles to form a block. It then is moved to a second chamber and so positioned that a piston compresses the block of curd at a 90° angle to the first, thereby causing the curd particles to knit more closely because of the lateral flow of the cheese in the block. The resultant block of cheese is relatively free from whey and air bubbles. R. Whitaker

292. Manufacture of cheese curd. E. W. Kielsmeier. U. S. Patent 2,851,363. 7 claims. Offic. Gaz., U. S. Patent Office, **734**, 2: 460. 1958.

The calcium and magnesium ions of milk are rendered inactive to delay coagulation when rennet and starter are added. After the rennet has acted on the milk, coagulation is induced by acidification with a food acid, and cheese is made in the usual way. R. Whitaker

293. Machine for packing dry cheese curd. H. Gold. U. S. Patent 2,850,047. 12 claims. September 2, 1958. Offic. Gaz., U. S. Patent Office, **734**: 1104. 1958.

Dry curd, such as cottage cheese curd, is moved into a chamber by a worm conveyor located in the bottom of a hopper. The chamber is of such size that the slightly compressed curd contained therein fills the containers to be filled when released to atmospheric pressure. A plunger discharges the contents of the chamber into the container on a conveyor beneath. The curd is confined in the chamber by a reciprocating gate constructed of a series of parallel prongs. R. Whitaker

294. Method of producing casein substantially free of vitamin B_{12}. G. Kissel. U. S. Patent 2,853,479. 11 claims. September 23, 1958. Offic. Gaz., U. S. Patent Office, **734**, 4: 1026. 1958.

Casein is rendered substantially free of vitamin B_{12} by heating a 1–10% solution of sodium, potassium, or ammonium caseinate at pH 5–7.4 to about 90 to 100° C. and adjusting to a pH of 4.49–4.51. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

295. Studies on the manufacture, storage, and uses of low-heat concentrated milk and skimmilk. P. E. JOHNSON, Okla. State Univ., Stillwater. Milk Prod. J., **49**, 9: 8. 1958.

A total of 36 trials, 25 with whole milk and 11 with skimmilk, was made in this study. Condensing ratios for the whole milk ranged from 2.23:1 to 4:1 (27.9 to 51.2% total solids) and condensing ratios for the skimmilk ranged from 3.16:1 to 5:1 (30.9 to 47.5% total solids). The forewarming temperatures were none, 130, and 140° F.

The low-temperature condensing apparatus used was of the recirculating type and was constructed of stainless steel. This apparatus was capable of handling from 4 to 6 lb. of finished product. The finished product was stored as quickly as possible at −10 to −14° F. Examinations were made in the fresh state and at 3 and 6 mo. of storage.

Results showed that some heat treatment of the milk was necessary to prevent lipolytic action. Oxidized flavor development in whole milk was the most serious problem. Upon reconstitution and pasteurization of the 3–6 mo. samples, it was often difficult to distinguish them from the natural, fresh product.

J. J. Janzen

296. Costs of processing, transportation, and distributing fresh or sterile concentrated milk. J. A. RIEKENS AND L. C. THOMSEN, Univ. of Wis., Madison. Milk Prod. J., **49**, 10: 6. 1958.

The authors recommend the use of Grade A milk for the production of concentrated milk.

Cost data were developed on the basis of 100 lb. original milk testing 3.76% fat, yielding 44.65 units (quarts of unconcentrated milk equivalent). On this basis, calculations showed that the cost to the grocer located in the area of production could range between $0.145 and $0.155, depending upon the operating margin desired.

Transportation costs are discussed in detail. The cost of transportation and warehousing to New Orleans would add $0.015 per one third-quart container. Thus, the price range to the grocer would be $0.16 to $0.17 per one third-quart of sterile concentrated milkk.

It is concluded that a good-flavored, concentrated milk would be able to complete, price-wise, with regular milk in milk-deficient areas.

J. J. Janzen

DAIRY CHEMISTRY

297. Chromatography of amino acids on sulfonated polystyrene resins. An improved system. S. MOORE, D. H. SPACKMAN, AND W. H. STEIN, The Rockefeller Inst. for Med. Research, New York 21, N. Y. Anal. Chem., **30**, 7: 1185. 1958.

A complete amino acid analysis of a peptide or protein hydrolyzate can be performed in 48 hr. with fraction collectors, or in 24 hr. with automatic recording equipment. The method can be used for determination of amino acids and related compounds in blood plasma, urine, and animal tissues. Columns of finely pulverized 8% cross-linked sulfonated polystyrene resins are used. The smaller particle size resin permits faster flow rates, and an appropriate choice of eluents has simplified operations. Amberlite IR-120 in "micropowder" form has shown good reproducibility from batch to batch over a 3-yr. test period.

B. H. Webb

298. A comparison of the Gerber with the Babcock and Mojonnier methods for testing butterfat in raw milk. W. F. LEWALLEN, Ky. Agr. Expt. Sta., Lexington. Milk Prod. J. **49**, 10: 14. 1958.

The Babcock, Gerber, and Mojonnier methods for testing fat in fresh raw milk and preserved milk that was stored for from seven to 15 days at 40° F. were compared. The results indicated that fat percentages obtained by the Gerber test were significantly higher than the Babcock and Mojonnier methods, at all periods of storage. The differences between the Babcock and Mojonnier methods were not significant.

J. J. Janzen

299. Specifications for Gerber glassware for determining the fat content of milk and cream. A. H. ROBERTSON AND C. E. HYRIDS, State Food Lab., N. Y. Dept. of Agr., Albany, N. Y. J. Milk and Food Technol., 21, 9: 259. 1958.

Revised specifications for the Gerber milk transfer pipette were suggested by the Netherlands Standards Commission on October 1, 1951.

The Gerber milk transfer pipette should be graduated to contain 10.77 ± .0.03 ml. H_2O at 20° C. The volume of the total graduated portion should be 1.00 ml. in the neck of the 8.0% milk test bottle, and 2.84 ml. in the neck of the 50%, 5-g. change cream test bottle.

H. H. Weiser

DAIRY ENGINEERING

300. Process of heating dielectrics by extended toroidal electrode. J. W. MANN AND G. F. RUSSELL. U. S. Patent 2,856,296. 10 claims. Oct. 14, 1958. Offic. Gaz., U. S. Patent Office, **735**, 2: 451. 1958.

Edible fluids are pasteurized by passage along a metallic toroid, along which a standing wave of radio frequency energy is maintained.

R. Whitaker

HERD MANAGEMENT

301. Device for measuring milk in a milking system. D. E. GOLAY. U. S. Patent 2,853,050. 3 claims. September 23, 1958. Offic. Gaz., U. S. Patent Office, **734**, 4: 917. 1958.

The reservoir on a milking machine consists of a spherical, transparent vessel with a flat bottom and a bail. When an observed volume of milk is to be discharged, the vessel is tipped and the milk allowed to pass out the suction line. R. Whitaker

MILK AND CREAM

302. Changes in the pH and the reduced ascorbic acid content of milk held for every-other-day pick-up in farm bulk milk tanks. B. J. LISKA AND H. E. CALBERT, Dept. of Dairy and Food Ind., Univ. of Wis., Madison. Milk and Food Technol., **21**, 9: 252. 1958.

The changes in pH and ascorbic acid content of raw milk held in farm bulk tanks and 40-qt. cans at 42° F. for 36 hr. were examined. Bulk tank milk lost 60% of the reduced ascorbic acid; milk held in cans lost 25%.

An increase of 10° F. in holding temperature of bulk tank milk caused a 10% increase in loss of ascorbic acid when the every-other-day pick-up was practiced. H. H. Weiser

303. Incidence of penicillin in the market milk supply of a local New England area. F. A. SHINO, R. E. CZARNECKI, AND W. K. HARRIS, Amherst Board of Health, Amherst, Mass. J. Milk and Food Technol., **21**, 8: 211. 1958.

Fifteen of 122 pasteurized milk samples from four milk distributors contained from .021 to 0.155 units of penicillin per milliliter when assayed by the ¼ in. disc method. A higher incidence of penicillin was detected when the ½ in. discs were used.

This survey indicates that one family in five consumed milk containing penicillin. These results are higher than those reported by many national surveys.

The ½ in. disc is preferable to the ¼ in. disc, because of convenience and higher sensitivity. H. H. Weiser

304. Time-saving mobile laboratory. Anon. Milk Prod. J., **49**, 10: 18. 1958.

The Connecticut Milk Producers' Association has been using a mobile laboratory for checking the accuracy of bulk milk storage tanks. The mobile unit is highly accurate and checks out tanks from four to eight times as quickly as was previously possible. The unit is equipped to carry its own water supply to whatever place is necessary. The water is pumped into the tank to be tested through a special meter that has a device for removing air and sediment. A program of constant rechecking of all producer tanks is now in progress. J. J. Janzen

305. Dyes as markers for antibiotics-contaminated milks. T. SMITASIRI, F. KOSIKOWSKI, R. GUTHRIE, AND M. FINCHER, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. J. Milk and Food Technol., **21**, 9: 255. 1958.

Four dyes were tested for effectiveness in detecting residual antibiotics in a milk supply. The dyes were annatto, Baker's red food dye, water-soluble chlorophyll, and fat-soluble chlorophyll.

Fat-soluble chlorophyll was rated as a good color indicator for detecting antibiotics in milk. When injected into the udder of treated animals, it could be detected over a period of ten milkings. This is roughly equivalent to the residual penicillin retention from a treatment up to 500,000 I. U. The dye did not interfere with the activity of the antibiotic and did not irritate animal tissue.

Although the use of this dye looks promising, it should not be accepted immediately because of the appearance of heavily stained particles in the milk which persist long after green color is depleted. The variability in retention of the antibiotics and/or the dye by different cows also is a factor to be considered.

H. H. Weiser

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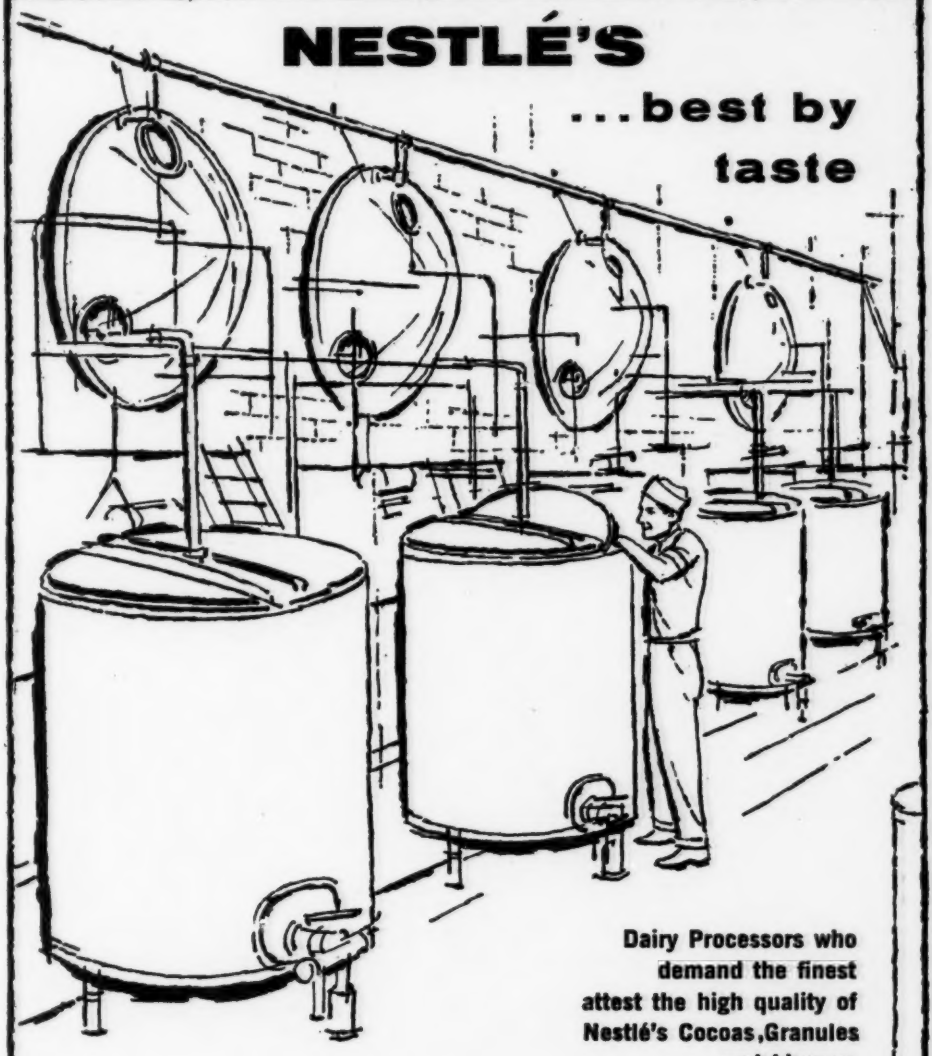

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